

A STUDY OF THE BIOLOGY AND ECOLOGY OF SELECTED PREDATORS OF
PHYTOPHAGOUS MITES ON FLORIDA CITRUS



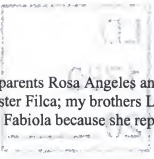
By

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This thesis is dedicated to my parents Rosa Angeles and Rafael M. Villanueva;
to my sister Filca; my brothers Luis; Julio; and Rafael; and
to Lucia Fabiola because she represents the next generation.

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Phytoseiid mites were found in higher numbers on leaves within the canopy of grapefruit trees than on outer leaves. No differences in phytoseiid abundance were found among leaf samples collected at different hours throughout the day between 0600-2200 h. Mite abundance on grapefruit trees was compared among young-leaves, healthy-mature leaves, and mined-leaves by *Phyllocnistis citrella* Stainton. Predacious phytoseiids and stigmatids and *Eotetranychus sexmaculatus* (Riley) were significantly more abundant on mined leaves than on young or healthy-mature leaves in weekly samples taken between February and April from an unsprayed orchard. *Eutetranychus banksi* (McGregor), and *Phyllocoptruta oleivora* (Ashmead) did not differ in abundance among the three types of leaves.

Development and reproduction of *Iphiseiodes quadripilis* (Banks) was evaluated on diets of *Malephora crocea* Jacquin and *Quercus* sp pollens and, *E. banksi*, *Panonychus*

citri (McGregor), and *P. oleivora* at 28 ± 1 °C, 14:10 (L:D) daylength and 45%-RH.

Iphiseiodes quadripilis completed its life cycle and oviposited viable eggs on exclusive diets of *M. crocea*, *Quercus* sp. pollens or *E. banksi* or *P. citri* eggs and motiles.

Panonychus citri stages were acceptable as prey to *I. quadripilis* but survival of larvae to adulthood was significantly lower than on *Quercus* sp. pollen. *Iphiseiodes quadripilis* larvae provided water, or *P. oleivora* alone, developed to protonymphs and then died.

Starved *I. quadripilis* nymphs and adult females preyed on *Aculops pelekassi* (Keifer),

but not on *P. oleivora*. *Iphiseiodes quadripilis* was significantly more abundant on

grapefruit leaves than on orange leaves in adjacent citrus orchards at four sites in central

Florida. This disparity was consistent whether samples were taken from border or

interior trees. Total numbers of phytoseiids were not significantly different between

citrus species in most cases. *Eutetranychus banksi* was significantly more abundant on

orange than on grapefruit, suggesting that biological control by *I. quadripilis* was better

on grapefruit trees. *Iphiseiodes quadripilis* preferred grapefruit leaves over orange leaves

in olfactometer experiments and in contact choice tests using leaf discs of the two citrus species.

Cryptothoea gloverii (Packard) (Lepidoptera) was identified as a predator of both *P. oleivora* and *P. citri*, although it also feeds on fruit and leaves of citrus. In the laboratory, *C. gloverii* preferred to feed on oranges russeted by *P. oleivora* than on fruit without russetting. *Feltiella* sp. and a *Lestodiplosis* sp. two undescribed Cecidomyiidae were highly specialized predators feeding on *P. oleivora* eggs, nymphs, and adults.

CHAPTER 1 INTRODUCTION

General Introduction

The citrus rust mite (CRM), *Phyllocoptruta oleivora* (Ashmead), and the pink citrus rust mite (PRM) *Aculops pelekassi* (Keifer) are the key pests of Florida citrus fruits (Childers and Achor 1999). These species are found in all citrus-growing regions of the world, especially in areas of high humidity. CRM was first recorded in Florida in the early 1880's (Hubbard 1883, Yothers 1914, Yothers and Mason 1930). Citrus rust mites cause direct damage to citrus by feeding on the epidermal cells of leaves, green twigs and fruit (Albrigo et al. 1981, McCoy 1976). Both the CRM and the PRM have probably been present in Florida since the middle 1800's but because of their small size, they were not properly identified until 1961 (Denmark 1963). Russetting of fruit surfaces reduces the value of fruit destined for the fresh market. Direct damage to fruit can also result in increased alcohol or phenol concentrations, reduction of fruit size, increased fruit drop, and increased water loss relative to undamaged fruit (Yothers 1918, Ismail 1971, Allen 1978, 1979, Yang et al. 1995). A positive correlation between CRM damage and the incidence of leaf infection by greasy spot, *Mycosphaerella citri* Whiteside, has also been observed (van Brussel 1975, Timmer et al. 1980), although both are favored by high humidity.

Acaricides are frequently applied for CRM control and expenditures range from 75-100 million dollars annually in Florida alone (Childers 1994a). Use of pesticides to

control rust mites is a temporary solution because CRM has developed resistance to several compounds such as zineb (Voss 1988, Childers 1994b) and dicofol (Omoto et al. 1995). CRM hormoligosis has been observed in response to various copper formulations (Childers 1994b). Similar phenomena have been reported for different mite pests in diverse cropping systems, i.e. *Panonychus ulmi* on apple (Villanueva and Harmsen 1998), *Tetranychus urticae* (Koch) on roses and tree fruits (Goka 1999), and on strawberry and pear (Croft et al. 1984). Other phytophagous mites of importance in the citrus orchard ecosystem of Florida include the Texas citrus mite (TCM), *Eutetranychus banksi* (McGregor), and the citrus red mite (CREM), *Panonychus citri* (McGregor). Although the latter species are more sporadic pests than CRM and PRM, in some instances, they require acaricide applications.

One possible way to break this cycle is through the use of selected pesticides that do not cause negative effects on natural enemies. Also, further research on natural enemies is needed to improve understanding of their ecological requirements and optimize their survival to the point where they might reduce CRM and PRM populations below economic levels. Predacious mites in the families Phytoseiidae and Stigmaeidae and certain predatory insects (cecidomyiid midges, neuropteran lacewings, coccinellid ladybeetles and several thrips species) have been identified as natural enemies that feed on citrus rust mites (Hubbard 1883, Muma et al. 1961, Peña 1992, Childers 1994a). While natural enemies of phytophagous mites in citrus are abundant, few have been well studied and their biology and behavior in relation to eriophyid (CRM and PRM) pests remain largely unknown. In this study, I examined various aspects of selected natural enemies of the eriophyid and tetranychid mites including their distribution, food sources,

host plant preferences and behavior. The natural enemies studied here included phytoseiid mites and insects (two cecidomyiids and a lepidopteran). The objectives pursued in this study were:

- 1) To evaluate the distribution of selected phytoseiid mite species between outer and inner leaves of the tree canopy to determine whether diurnal migration within the tree occurs (Chapter 2).
- 2) To compare the numbers of predacious and phytophagous mites between leaves damaged by *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), healthy mature leaves and young leaves. Also, the relationship between numbers of phytoseiids and the presence of pollen grains on citrus leaves was examined (Chapter 3).
- 3) To evaluate the development and reproduction of *Iphiseiodes quadripilis* (Banks) on different diets in the laboratory and determine the acceptability of the citrus rust mite and the pink citrus rust mite (PRM) as food sources for different *I. quadripilis* life stages (Chapter 4).
- 4) To determine if *I. quadripilis* expresses a preference for grapefruit leaves over sweet orange leaves. (Chapter 5)
- 5) To characterize two cecidomyiid midge species and a predatory moth, *Cryptothoelea gloverii* (Packard) (Lepidoptera: Psychidae), that have been observed preying upon citrus rust mite and describe their ecological relationships with pest mites and citrus plants (Chapter 6).

Review of Literature

A diverse fauna of predacious mites inhabit Florida citrus orchards including at least 33 different species of Phytoseiidae (Childers and Denmark, unpublished). Although these mites are important natural enemies of many pests within the citrus ecosystem, their role in controlling phytophagous mites is poorly understood. Many of these predators feed on pollen, some are generalist predators, and other species may have little or no association with pest mites (Muma et al. 1961, McMurtry 1977, Peña 1992, Childers 1994a). Furthermore, insects such as coccinellids, chrysopids and cecidomyiids have been described as predators of phytophagous mites, further complicating the ecological relationships between pest mites and their predators in the citrus ecosystem.

The economically important tetranychid mites on Florida citrus include the citrus red mite (CReM) *P. citri* and the Texas citrus mite (TCM) *E. banksi*. Both TCM and CReM prefer dry conditions and are more abundant in the citrus-growing regions of California and Texas, respectively than in Florida. Both spider mite species can emerge as important pests in Florida from March through June (Childers et al. 1991; Childers 1994a). TCM has a tan to brownish-green color with dark green to black spots on the lateral sides of the body and its eggs are flat and vary from yellow to green in color. All instars of CReM and eggs are reddish in color. Both mites feed on green twigs and fruit and cause stippling on the leaves and mesophyll collapse (Albrigo et al. 1981). When TCM feeding injury follows dry weather conditions in Texas, it may result in leaf drop in the tops of citrus trees (Dean 1980). TCM is presently the most common spider mite on Florida citrus of these two spider mites (Childers et al. 1996).

CRM and the PRM mite are the most abundant and important eriophyid species on citrus in Florida (Childers 1994a, Childers and Achor 1999). The citrus bud mite (CBM) *Aceria sheldoni* (Ewing) also occurs in Florida but is not an important pest (Childers and Achor 1999). In contrast, *A. sheldoni* is an important pest in California. Denmark (1963) provided a key to separate these three eriophyid mite species. CRM has been reported on Florida citrus since the mid 1800 (Yothers and Mason 1930). Both CRM and PRM are found in all citrus-growing areas of Florida (Childers and Achor 1999).

CRM and PRM are frequently misidentified, partly due to their small size. However, differences in their phenology, oviposition sites, body shape and coloration have been observed. PRM migrates earlier than CRM from leaves to fruit, sometime between April and May (Childers and Achor 1999). CRM eggs are spherical and tend to be deposited in depressions on the fruit surface including the oil glands, whereas PRM eggs are translucent and are deposited indiscriminately on the fruit surface (Childers and Achor 1999). CRM have a convex ophisthosoma (dorsal area) and CRM have a concave ophisthosoma (Denmark 1963). Body coloration is yellow to straw for the CRM and pink to reddish for the PRM, although this character alone is not conclusive for distinguishing the species. CRM and PRM feeding results in direct damage to epidermal cells of leaves, green twigs and russetting on the rind surface of fruit (Allen 1979, Albrigo et al. 1981, McCoy 1996). Heavily damaged fruit are discolored and have a reduced value in the fresh market (Albrigo and McCoy 1974).

The conventional method to control citrus rust mites is with acaricides (Childers et al. 1996). Hubbard (1885) reported the application of sulphur powder to control CRM but this had negative impacts on phytoseiids and other natural enemies. Fungicides used for

control of pathogens also have been used to control citrus rust mites but resistance to many of these products has developed (Childers 1990). In addition, fungicides have a negative impact on the pathogenic fungus *Hirsutella thompsoni* Fisher, that attacks citrus rust mites at high population densities (Fisher and Griffiths 1950). Petroleum oils in combination with certain insecticides or acaricides (i.e. abamectin and ethion) also have been used to control rust mites and these mixes improved the residual activity of the acaricides (Childers et al. 1996). However, petroleum oil applications initially were restricted from the mid-summer to mid fall period due to tree and fruit injury factors (Carman 1977). New oil formulations with additives such as oxidation inhibitors prevented phytotoxicity (Kipiani 1976). Today, oil sprays are becoming increasingly important in integrated pest management on Florida citrus (Childers et al. 1996) and other parts of the world: Matsumoto et al. (1981a, b) in Australia and Japan, Walker and Aitken (1996) in California, Rae et al. in China (2000). Factors such low mammalian toxicity, low cost, and the additive residual activity when combined with certain acaricides, contribute to petroleum oil use. Further CRM has developed resistance to pesticides such as: diflubenzuron (Knapp et al. 1988), zineb (Voss 1988), fenbutatin oxide (Childers 1994b), dicofol (Omoto et al. 1995), fenpropathrin (French and Villarreal 1990). On the other hand, natural enemies (phytoseiid) populations are significantly lower in orchards that received heavy sprays of acaricides, insecticides and fungicides. Pesticides also induced resurgence of CRM populations (Voss 1988, Childers 1994b); due to hormoligosis (Dittrich 1987), and potentially leading to a pesticide treadmill effect. Moreover, costs for chemical control of citrus rust mites are at least 75 million dollars annually in Florida (Childers 1994a) but may be as high as 100 million (McCoy 1996).

In addition to these pests, there are phytophagous mites that are considered of secondary importance and include the six-spotted mite (6SM), *Eotetranychus sexmaculatus* (Riley), the two-spotted spider mite (2SSM), *Tetranychus urticae* Koch, the broad mite, *Polyphagotarsonemus latus* (Banks) (Tarsonemidae), and a species complex in the genus *Brevipalpus* (Tenuipalpidae). These pest mites can cause serious localized damage in citrus orchards or in greenhouses on occasion. Special attention should be given to the *Brevipalpus* species because they transmit citrus leprosis virus in citrus and cause considerable losses in those countries where the disease occurs. The virus does not occur in Florida today (Childers et al. 2001) but it has been previously reported and referred to as nailhead rust or scaly bark in Pinellas County (Fawcett and Lee 1926)

Phytoseiid mites are considered effective biological control agents of spider mites in numerous cropping systems (grapes - Baillod *et al.* 1982; apples- Childers and Enns 1975, Bostanian and Coulombe 1986; greenhouses – Sabelis 1985a and Brodsgaard and Hansen 1992, De Moraes and McMurtry 1986; strawberry - Easterbrook 1998; cassava - Yaninek et al. 1998). There have been several studies on phytoseiid mites on Florida citrus, but the abundance, biology and predatory capacity of several species are inconclusive or unknown. Muma (1964) reported the abundance of phytoseiid mites on citrus leaves in a survey on north, central and south Florida as follows: *T. peregrinus* (= *Amblyseius peregrinus*) 62.9 %, *I. quadripilis* (= *Amblyseius quadripilis*) 15.3 %, *Typhlodromips simplicissimus* (De Leon) (= *Amblyseius simplicissimus*) 5.3%, *Euseius hibisci* (Chant) (= *Amblyseius hibisci*) 4.5%, *Typhlodromina subtropica* Muma and Denmark (= *T. conspicua*) 2.9 %, *Galendromus alveolus* (= *G. floridanus*) 2.8 %, and 10 other phytoseiid species represented 6.3 %. Peña et al. (1989) sampling for predators of

P. latus in southern Florida lime trees found that *T. peregrinus* accounted for 72 % of all the predacious mites followed by *T. dentilis* (15%). Childers (1994a) reported that the most abundant phytoseiid mite on citrus was *Euseius mesembrinus* (Dean), followed by *Typhlodromalus peregrinus* (Muma), and *Iphiseiodes quadripilis* (Banks). The discrepancy between Childers (1994), and Muma (1964) and Peña et al. (1989) on the abundance of *E. mesembrinus* and *T. peregrinus* can be due to sampling location and/or time differences. Also, *E. mesembrinus* is a relatively new species in Florida, and first reported by McCoy and Rakha in 1985.

Research on *E. mesembrinus* has demonstrated that it preys on tetranychids and also completes its life cycle on the pollen of various plants (Abou-Setta and Childers 1987, Badii and Hernandez 1993). However, studies using eriophyids as prey have produced unsatisfactory results. Abou-Setta (1988) stated that *P. oleivora* was not a suitable prey for *E. mesembrinus*. Badii and Flores (personal communication, Universidad Autonoma de Nuevo Leon, Mexico) could not rear *E. mesembrinus* on CRM alone, and even when *E. mesembrinus* was starved for 24 h and then placed in arenas with CRM, predation was not observed (personal observation). Muma (1967a) noted that *T. peregrinus* reproduces and develops on *P. citri*, *E. sexmaculatus*, *E. banksi*, white fly eggs and *Parlatoria pergandei* (Comstock) eggs and crawlers. Peña (1992) showed that adult *T. peregrinus* did not reject *P. oleivora* as a prey when it was provided as a food source but favored *Polyphagotarsonemus latus* when the latter was present. Fouly et al. (1995) reported that *T. peregrinus* completed its life cycle on diets of *T. urticae*, *P. citri*, and pollens of *Malephora crocea* Jacquin (ice plant), *Quercus* spp. (oak) and *Typha latifolia* L. (cattail).

The shiny button mite (SBM), *Iphiseiodes quadripilis* is presently the third most abundant phytoseiid on Florida citrus (Childers and Denmark, unpublished data). The SBM has been described as a facultative generalist predator and it can feed on eriophyids, tetranychids, honey alone, citrus pollen and other less economically important citrus mites (Muma, 1971). However, little is known about the life cycle of SBM, its predatory behavior, or the ecological factors that influence its development. This is the least studied phytoseiid of the three previously discussed. The SBM can be easily recognized with the naked eye and it is distinguishable from other phytoseiid mites by its shiny, dark, mahogany-red color and its round, almost hemispherical body shape as an adult (Muma 1975). SBM can be misidentified with the velvet mite *Phytoscutus sexpilis* (Muma) in Florida, because the latter has a velvety, rose-red body color and similar body shape. However, under the dissecting stereomicroscope, four long slender setae project from the distal end of the body whereas the SBM has only two setae projected backwards (Muma and Denmark 1971).

Stigmaeid mites are predacious on many pest mites but are less abundant than phytoseiids in citrus orchards (Childers 1994a). Muma et al. (1961) described five species of Stigmaeidae, Rakha and McCoy (1984) described an additional species, and recently Ueckerman and Childers discovered two more (personal communication). *Agistemus floridanus* Gonzalez is the most common and feeds on *P. citri*, *E. sexmaculatus* and *Tydeus gloveri* (Ashmead) (Tydeidae). *Agistemus floridanus* also feeds on CRM and *A. floridanus* adults were maintained on an exclusive diet of CRM alone by Muma and Selhime (1971). This report, although largely descriptive and without statistical analysis, indicated that *A. floridanus* had a long generation time and a

low reproductive rate. Yue and Childers (1994) noted that the stigmatid *A. exertus* Gonzales found on citrus had a lower prey consumption rate when compared with the phytoseiid *Phytoseiulus persimilis* Athias-Henriot. The significance of stigmatids is discussed here because these predacious mites are important within the citrus acarine system and can prey on pest mites. Their relationship with phytoseiids has not been studied. Stigmatids are commonly found in the apple orchards of southern Ontario and northeastern New York and the stigmatid *Zetzellia mali* (Ewing) contributes to the biological control of the apple rust mite, *Aculus schlehdentali* Nalepa and the European red mite, *Panonychus citri* Koch (Villanueva 1997). Also, *Z. mali* feeds on the eggs of the phytoseiid *Typhlodromus caudiglans* (Schuster), but phytoseiids were unable to consume *Z. mali* eggs (Clements and Harmsen 1990). MacRae and Croft (1996) found a similar behavior with *Z. mali* in Oregon although in this case the phytoseiids *Metaseiulus occidentalis* Nesbitt and *Typhlodromus pyri* (Schueten) consumed a cumulative mean of < 1 *Z. mali* egg or immature after 6 days. Both phytoseiids and stigmatids can contribute to pest mite control under high prey densities. The competitive relationship between these two families of predacious mites was described in apple orchards (Clements and Harmsen 1990) and can occur on Florida citrus.

The acarine fauna on citrus orchards in Florida includes a diverse group of species from different families. Most do not damage citrus and their potential capacity as natural enemies is poorly understood. These mites include different species in the following predacious families: Anystidae, Bdellidae, Eupalopsellidae, Cheyletidae, and Cunaxidae. Also, there are mite species that can be an alternative prey. The latter group includes the

Tydeidae, Tarsonemidae, and Acaridae (fungus mites). These two groups have not been well studied and their relationships with phytoseiids are unclear.

McMurtry (1992) noted that generalist phytoseiids predominate in citrus in California. These species consume a variety of foods, including some of plant origin such as pollen (McMurtry and Croft 1997). It is possible to hypothesize that the feeding and attacks on CRM by phytoseiids may be lessened due to: 1) the presence of alternative prey that are larger or more abundant than CRM. This alternative prey present on Florida citrus orchards includes tetranychids, tydeids, tarsonemids and fungal mites. 2) The alternative prey also can be a more suitable prey and enhance phytoseiid reproductive capacity; and 3) generalist phytoseiids rather than specialist predators are more abundant in citrus, which was noted by McMurtry (1992) in California. McMurtry and Croft (1997) defined 'generalists' as those species that consume a variety of foods including some of plant origin such as pollen in some perennial plants. Two of the most abundant phytoseiids of Florida, *E. mesembrinus* and *T. peregrinus* fall within this category. Either one or all of these factors may contribute to lessen the impact of phytoseiids on eriophyids in Florida citrus.

In addition to the mite and insect predators of phytophagous mites described above, the fungus *H. thompsonii* was first reported by Yothers and Mason (1930) in association with *P. oleivora*. Subsequent evaluations of *H. thompsonii* have been made by several authors (Muma 1955a, McCoy et al. 1971, McCoy and Couch 1982). However, to be effective *H. thompsonii* requires high CRM population densities and high relative humidity to reach epizootic levels. McCoy (1985) was unable to reduce CRM populations by spraying *H. thompsonii* formulations.

Insects also contribute to reducing phytophagous mite populations and four orders of insects are known to prey on CRM on citrus. Hubbard (1883) found a midge larva (Diptera: Cecidomyiidae, formerly Itonididae) feeding on CRM and later Muma et al. (1961) collected *Itonidini* species within *P. oleivora* infestations. Yothers and Mason (1930) described *Stethorus nanus* LeConte (Coleoptera: Coccinellidae) and *Chrysopa* sp. (Neuroptera: Chysopidae) preying upon CRM. Muma (1958, 1967a; Muma et al. 1961) also mentioned *Coniopteryx vicina* Hagen (Neuroptera: Coniopterygidae) preying upon CRM. The thrips *Scolothrips sexmaculatus* (Pergande) and *Leptothrips mali* (Hunter) (Thysanoptera) have been reported as predators of CRM by Bailey (1939) and Muma (1955b), respectively. This study focuses only on cecidomyiids and *Cryptothelea gloverii* (Packard) (Lepidoptera; Psychidae) found on citrus and their predacious behavior toward the citrus rust mites.

The genus *Feltiella* has a worldwide distribution (Gagne 1989) and all described species form a distinctive group associated exclusively with spider mites (Tetranychidae) Gagne (1995). *Feltiella acarisuga* (Vallot) has been reported preying on tetranychid mites in various crops such as vegetables (Opit et al. 1997, Gillespie et al. 2000), and strawberries (Croft et al. 1998, Easterbrook 1998, Enkegaard et al. 2000). However, there are few examples of predation by cecidomyiids on eriophyid mites. The eriophyid *Aceria litchii* Keifer is a serious pest of *Litchi chinensis* Sonnerat (Sapindaceae) in Australia and China and is preyed on by a cecidomyiid larva *Arthrocnodax* sp. (Waite and Gerson 1994). *Medetera* species (Diptera: Dolichopodidae) have been observed preying on *Aculus schlechtendali* Nalepa on apple in Washington (Rathman et al. 1988). Cecidomyiids have been reported feeding on CRM in California (Hubbard 1883) and

Florida (Muma et al. 1961) but these reports were vague and the dipteran species were not identified.

The orange bagworm (OBW) *Cryptothoele gloverii* (Packard) (Lepidoptera: Psychidae) was first described in Florida in 1858 (Hubbard 1885). The larvae of bagworms can be easily recognized by their portable case or bag in which they reside. It is constructed of dense silk and then covered with bits of dry leaves, bark and other scraps (Quayle 1938). The bag has an irregular conical shape and, when the larva is fully grown, it is approximately 1.8 and 1.3 cm long for females and males, respectively (Hubbard, 1885). Hunt (1923) described an outbreak of OBW on orange in Sebring, Florida, and reported damage on fruit, growing twigs, and mature leaves. The OBW was also described as a predator of the camphor scale *Pseudaonidia duplex* Cockerell, the soft brown scale *Coccus hesperidum* L., a species of *Ceroplastes*, and black scale *Saissetia olea* (Olivier) (Plank and Cressman 1933). No further information on this insect is available on citrus.

CHAPTER 2

PHYTOSEIID DISTRIBUTION ON LEAVES SAMPLED THROUGHOUT THE DAY AND EFFECTS OF LEAF POSITION IN THE CITRUS CANOPY

Introduction

The small size of predatory phytoseiids (and many other mites i.e. stigmatheids) is a factor that makes mite field studies more laborious. In addition, phytoseiids are scarcer than their prey, but they are more active and faster than stigmatheids (Muma and Selhime 1971, Chant 1985). These predators are continuously moving when they are foraging for prey or food (Sabelis 1985b). This foraging behavior, however, may not only depend upon prey availability but also on abiotic factors such relative humidity, temperature and light intensity. Muma (1967b, 1975) observed that *T. peregrinus* is active at night and prefers shaded leaves during the day. Allen and McCoy (1979) also noticed that the distribution of citrus rust mites on individual fruit and in the whole citrus tree suggests an avoidance of direct sunlight. Villanueva and Childers (unpublished) also noticed analogous responses of *P. citri* and *E. banksi* on citrus. Other researchers studying other species of phytoseiids (C.C. Childers; R. Harmsen; personal communications) have observed similar behavior.

This type of behavior can be described as negative photo-taxis (Fraenkel and Gunn 1961) and may have evolved as a defense mechanism against high temperatures and low humidity on external leaves during daylight hours. Indeed, the large body surface area and small size of these mites results in increased water loss potential when exposed to

light. Therefore, phytoseiids may find better micro-environmental conditions on internal leaves rather than on outer leaves (Allen and Syvertsen 1981). Time of the day for sampling may influence phytoseiid counts because most of the orchards in Florida are set north-south (Wheaton et al. 1978). This is because phytoseiids may be affected by the direct incidence of sunlight that can increase the temperature of the eastern leaves in the morning and the western leaves in the afternoon. Hence phytoseiids can migrate from the east to the interior leaves in the morning and in the afternoon they may return when these leaves become shaded. Similar movement of phytoseiids may occur on the western side but in the opposite direction.

Temperature and relative humidity in the interior leaves of the tree canopy are more constant compared with conditions on the outer leaves (Ferro et al. 1979) and these factors indubitably affect mite populations. Moreover, we must consider the existence of a boundary layer on fruit or leaf surfaces. The boundary layer is a thin space above the leaf surface up to 1000 μm deep, where temperature, and %RH are more constant than the surrounding environment (Allen and Syvertsen 1981). The thickness of the boundary layer decreases with high winds (Gates 1968). This boundary layer is the result of leaf or fruit transpiration and provides unique micro-environmental conditions on the surface with different gradients of temperature and humidity than their surroundings. Small mites such as eriophyids are able to survive and flourish in this highly localized microenvironment. The boundary layer also provides resistance to water vapor diffusion and resistance to heat transfer between a leaf and its surroundings (Martin et al. 1999). The east side of the tree canopy receives direct solar radiation during the morning hours; whereas the west side of the tree canopy is affected in the afternoon. Solar radiation

would be significantly different in small body sizes and it may be possible that this affects phytoseiid densities depending on how the solar radiation is directed. The aim of this chapter is to determine whether there are any differences in phytoseiid populations on the east, west or interior leaves of the tree canopy depending on time of sampling. In addition, the distribution of phytoseiids within the tree canopy was assessed to determine if time of sampling resulted in phytoseiids density changes.

Materials and Methods

This experiment was conducted in a grapefruit orchard in Highland City, Polk County, Florida. The trees were 20 years old and the orchard was selected because of the occurrence of high numbers of phytoseiid mites based on previous experiments. The trees were minimally sprayed. Four 'Marsh' grapefruit trees were selected, flagged and marked. All trees were in the same row and separated from each other by 8 to 10 trees. Leaves were collected randomly from the tree canopy between 1 and 2 m. per sample type. Forty leaves were collected from each of the interior, east and west sides of each of four trees. Each 40-leaf sample was placed in an individual labeled jar containing between 0.2 to 0.3 liters of 80% ethanol and taken to the laboratory. In the laboratory, leaves were rinsed in water over a No. 400 sieve with an average opening of 38 μm . The residues including mites, small organic particles, soil and spider webbing remaining in the sieve were placed in separate labeled jars with 80% ethanol for each sample. Later all of the phytoseiids found in each jar were sorted and counted using a dissecting stereomicroscope. The phytoseiids from each sample were then placed in a small vial containing 80% ethanol and labeled for eventual slide preparation. Mites were slide-mounted in Hoyer's medium (Krantz 1978), oven dried and later identified to species.

Samples were collected on 14, 20 and 28 October 1999 at 2 h intervals (0600, 0800, 1000, 1200, 1400, 1600, 1800, 200 and 2200 h). Similar types of samples were collected on 9 and 16 March and on 17 and 24 August 2000. However, in the last two months, the leaves were collected at 3 h intervals (0600, 0900, 1200, 1500, 1800 and 2100 h.). All dates were selected when the weather forecast indicated clear and sunny days with low probabilities of rain.

To record environmental conditions, three HOBO® data loggers (Onset Computer Corporation, Bourne, MA) were used during each of the sampling dates and placed on the east, west and interior parts of the tree canopy. The HOBO data loggers registered temperature in Celsius degrees, % relative humidity and light intensity in lumens.

Phytoseiid data were transformed as $\sqrt{(X + 0.5)}$, where X is the number of phytoseiids found on each 40-leaf sample. A two-way analysis of variance was performed on all data followed by Tukey's Honest Significant Difference (HSD) test for separation of means using Statistica ® (Stat Soft, Inc. 2000). Figures presented show original mean values of data without transformation \pm standard error of means (SEM).

Results

Cultural practices such as disking, hedging, mowing, application of fertilizers and pesticides for 1999 and 2000 are presented in Table 2.1. Environmental conditions for the sampled dates are presented in Fig. 2.1. Environmental data for August showed that mean %RH inside the tree canopy was 7 to 11% higher than the outside canopy (east and west) %RH. Temperatures were 3.3 to 4.7° C lower inside the tree canopy than outside and the light intensity was 400 lumens lower on the shaded side versus the sunny side of

the outer canopy. Environmental data for October 1999 and February 2000 showed similar trends (Fig. 2.1). The environmental conditions inside the canopy might be more favorable for phytoseiid development than the outer canopy.

The two-way analyses of variance for all the sampled dates are presented in Table 2.2. Position of leaf sampling (east, west, or inside tree canopy) showed significant differences ($P < 0.05$) in the number of phytoseiids but effects of sampling time throughout the day and the interaction were not significant on any date ($P > 0.05$). Grouping the total number of phytoseiids found for all the three dates in October, 1999 yielded 346, 366, and 248 phytoseiids in each of the 1,440 leaf samples. Similarly, for March, 2000 the numbers were 599, 1,964, 613 on the east, inner and west sides, respectively, for each of the 960 leaf samples. For August 2000, the numbers were 69, 435, and 37 on the east, inner and west side of the tree canopy, respectively, for each of the 960 leaf samples. More phytoseiids were collected in the March 2000 samples (mean = 1.10 phytoseiids/leaf) than in October 1999 or August 2000 (mean = 0.16 and mean = 0.19 phytoseiids/leaf, respectively).

No significant differences were obtained for any of the samples in October 1999 using Tukey's honest significant differences (HSD) test (Fig. 2.2). In contrast, samples taken in March 2000 resulted in the highest numbers of phytoseiids collected (Fig 2.3). Samples taken on 16 March at 1200 h had the highest number with 60 ± 14.12 (mean number \pm SEM) phytoseiids /40 leaves. Significantly higher numbers of phytoseiids ($P < 0.05$, Tukey's HSD test) were obtained from the inner leaves than from either east or west leaf samples at 1200,1500,1800 and 2100 h on 9 March 2000 and during all the times throughout the day on 16 March (Fig. 2.3) using HSD Tukey's test. The numbers

of phytoseiid counts in the August samples were very low. However, the trend was similar to the March samples. Most of the predators were found on the inner than outer leaves (Fig. 2.4). Significant differences were found ($P < 0.05$, Tukey's HSD test) at 0600, 1200, and 2100 h on 17 August; and again at 0600, 1200, 1500 h on 24 August.

The phytoseiid species found in the orchard were: *Typhlodromalus peregrinus* ($n = 870$), *Iphiseiodes quadripilis* ($n = 282$), *Euseius mesembrinus* ($n = 184$), *Typhlodromina subtropica* Muma and Denmark ($n = 21$), *Typhlodromips dentilis* (De Leon) ($n = 20$), *Clavidromus transvaalensis* (Nesbitt) ($n = 2$), *Galendromus helveolus* ($n = 1$), *Typhlodromalus* sp. ($n = 1$) and *Phytoscutus sexpilis* ($n = 1$). Their distributions in the tree canopy are presented in Fig. 2.5. The most abundant species were *T. peregrinus* (42.1%) and *I. quadripilis* (50.4%) on October 1999. The most abundant species was *T. peregrinus* (76.2%) in March 2000 and, *E. mesembrinus* was the most abundant (54.1% of all identified species) in August 2000.

Discussion

The relative abundance of phytoseiids during the three periods of sampling (October 1999, March 2000 and August 2000) was dissimilar and the cause of this can be explained by the phenology of these mites. Generally phytoseiid species are more abundant from March to May (Childers 1994a). Muma (1967b) referring to *T. peregrinus* (then the most abundant phytoseiid in Central Florida) stated that its population was larger during late winter and early spring. This coincides with the data presented here and also can be explained by the presence of pollens (*Citrus* sp., *Quercus* sp, *Pinus* sp., and various weed species) that are abundant in March (see Chapter 3).

No significant differences in abundance of phytoseiids ($P>0.05$) were obtained for the sampling times throughout the day. This latter result is unexpected because the initial hypothesis for sampling throughout the day was that direct solar radiation on the east sided leaves during the morning would initiate a migration of phytoseiids from the east to the inner leaves. Also, it may be possible that phytoseiids would be foraging on the west side because this direct sunlight increases temperatures and reduces %RH on the east side. Likewise the opposite would happen on the west side; west sided leaves would have fewer mites at mid afternoon than the eastern leaves for similar reasons. This result can be explained by the preference of phytoseiids for the lower leaf surface and the boundary layer of the leaf surface that acts like a buffer producing more stable environmental conditions. In addition, phytoseiids can also rest in crevices and bark of the citrus trunk during times of high solar radiation. Despite the solar radiation the east-sided leaves received during the morning, the environmental conditions between these leaves and the west-sided leaves might be similar within the space enclosed by the boundary layer compared to their surroundings (Gates 1968, Ferro et al. 1999). Still, there are studies that reported phytoseiid differences in sampling times throughout the day in citrus. Garcia Mari et al. (1985) reported that *Euseius stipulatus* ((Athias-Henriot) was more abundant at night between 2200 and 2400 h than sampling during the daylight on outer leaves of 'Washington' navel in Spain. Muma (1967b) also described a similar nocturnal abundance of *T. peregrinus* in Central Florida. Garcia Mari et al. (1985) and Muma (1967b) explained this behavior of phytoseiids as a search for prey. Phytoseiids moved from mature inner leaves to the outer young leaves at night because these leaves had more tetranychid prey. McMurtry et al. (1970) explained this behavior as a search

for shaded areas and Ferragut et al. (1988) explained that phytoseiids preferred trees with abundant foliage. On other plants, phytoseiids also used domatia structures as a refuge site (O'Dowd and Willson 1997, Walter 1996, Roda et al. 2000, Norton et al. 2001). Recently, Onzo et al. (2002 In Press) observed that the phytoseiid *Typhlodromalus aripo* De Leon rested in domatia structures during the day on cassava and searched for the green cassava mite *Mononychellus tanajoa* (Bondar) on leaves at night. Domatia do not exist on citrus leaves but phytoseiids use mines created by the leafminer *Phyllocnistis citrella* Stainton (Gracillariidae) in a similar way to domatia structures since they provided shelter to the predator (see chapter 4).

No significant differences ($P > 0.05$) were obtained for the interaction between sampling hours and sampling side (Table 2.2). However, significant differences ($P < 0.05$) occurred with the two-way ANOVA on the numbers of phytoseiids obtained in sampling position (east, inside or west side of tree canopy) for all dates (Table 2.2), but these differences did not occur ($P > 0.05$) with the Tukey's HSD post-hoc test of means for all dates sampled on October 1999 (Fig. 2.2). The highest number of phytoseiids found during October was 10.0 ± 1.43 (mean \pm SEM) motiles/40 leaves from samples collected on 20 October at 0800 h (Fig. 2.2). This number is very small (0.25 phytoseiids/leaf) if we extrapolate the number to phytoseiids/leaf. Hence the low number of phytoseiids found during this month produced greater variability between the sampling sides and this reduced the probabilities to observe differences. These lower phytoseiid numbers may be affected negatively by the herbicide glyphosate (Roundup ®) and the insecticide chlorpyrifos (Lorsban ®) sprayed on 13 September 1999 just one month before the October 1999 sample and the petroleum oil spray on 17 July 2000 one month

before the August 2000 sample. Chlorpyrifos is relatively non-toxic to *E. mesembrinus* but has a repellent effect and petroleum oils are moderately toxic to non-toxic to this phytoseiid species (Childers et al. 2001).

The samples collected in March and August 2000 showed that higher numbers of phytoseiids were present on the inner leaves (Figs. 2.3 and 2.4) than the outer leaves of the tree canopy. Significant differences ($P > 0.05$, Tukey's HSD test) were found for many of the hours sampled. In March, this is apparent because the numbers of mites were the highest observed (0.6, 2.0, 0.6 phytoseiids/leaf on the east, inner and west side of the tree canopy, respectively). Although phytoseiid numbers in August were lower than in March, the phytoseiids showed a similar trend. More phytoseiids were found in the inner canopy compared to leaves collected from the east and west sides (0.4, 0.07 and 0.03 phytoseiids/leaf, respectively). Phytoseiid numbers on the inner leaves showed considerable variance but were significantly different ($P < 0.05$) when compared to outer leaf samples mainly because the outer east and west leaves of the tree canopy were very low (Figs. 2.3 and 2.4). This latter observation can be explained as an adaptation of phytoseiid mites to inner leaves that work like a refuge, protecting them from harsher environmental conditions that occur on outer leaves exposed to higher temperatures and lower humidity conditions (Fig. 2.1). Muma (1967b) observed similar results with *T. peregrinus*. However, he provided only mean numbers without statistical analysis or time of the year that experiments took place.

Although Fig. 2.5 can be misleading because it is expressed in percentages; numerically the most ubiquitous and abundant phytoseiid was *T. peregrinus* during the three month sampling periods. Also, in Fig. 2.5 it is evident that both *I. quadripilis* and

T. peregrinus were equally abundant in October 1999, and *T. peregrinus* was the most abundant in March 2000, whereas *E. mesembrinus* was the most abundant phytoseiid in August 2000. Muma (1964) observed similar density patterns in the winter and summer months with *T. peregrinus*. It is possible that the observed densities of *E. mesembrinus* were due to the mite's phenological cycle. There is not enough evidence to confirm this categorically due to the low numbers observed in the summer months. However, *I. quadripilis* was found throughout the year (Muma and Denmark 1971) and this corresponds to the results observed here. Childers and Denmark (unpublished) found similar seasonal abundance trends with these species, although they noted (personal communication) that *E. mesembrinus* was most abundant followed by *T. peregrinus* and *I. quadripilis*. Their study also was more extensive and intensive. It included 7 orchards in central and south Florida and there were monthly samples taken from 1994 to 1996. Data presented here however, coincides with Peña et al. (1989). They found that *T. peregrinus* was the most abundant phytoseiid in southern Florida with 72.4 % although the total numbers of predators identified were only 472. The rest of the phytoseiid species identified are found in Florida although they are less common and poorly studied (Muma 1964, Muma and Denmark 1971).

March and August 2000 (Figs. 2.3 and 2.4) data have shown that phytoseiid mites are more abundant on the inner leaves of the tree canopy. However, numbers of phytoseiids obtained in October 1999 (Fig. 2.2) were low, and this may be the reason why significant differences were not observed. Phytoseiid abundance on Florida citrus is generally observed during the months of March to May. This is one reason why the March data resulted in higher phytoseiid numbers and not to the sampling technique employed. This

peak of phytoseiid abundance coincides with the pollen season of orange and grapefruit and several trees (oak, pines and various weed species) and with tetranychid mites abundance in Florida. An explanation to the increase in the phytoseiid populations between March and May is the presence of abundant pollen during these months. Evidence of pollen feeding by the three most abundant phytoseiids was presented in studies by Abou-Setta and Childers (1987) on *E. mesembrinus*, Peña (1992) on *T. peregrinus* and in chapter 4 for *I. quadripilis*. All three species completed their life cycle and reproduced on different pollen diets alone in the laboratory. From results presented here we confirm that sampling time through the day did not affect phytoseiid densities and that there is no evidence that phytoseiids move from the inner leaves to the outer leaves during the night. However, the data presented confirm that phytoseiids prefer inner leaves compared to outer leaves of citrus. This can be due to more adequate micro-environmental conditions (temperatures and relative humidity) in inner than outer leaves.

Table 2.1 Agricultural practices employed in the Bader grove in Highland City.

Date	Material / Practice	Product Name	Rate / Hectare
1 Mar 1999	Disking 1 way	—	—
24 Mar 1999	Hedging	—	—
10 May 1999	Herbicide spray	Glyphosate	2.8 l
	Herbicide spray	Simazine	4.7 l
	Insecticide	Chlorpyrifos	0.47 l
30 May 1999	Fertilizer	Techmangam	14.0 Kg
		Zinc	7.0 Kg
	Insecticide spray	Soluble oil	58.4 l
28 Jul 1999	Disking 1 way	—	—
31 Jul 1999	Fungicide spray	Soluble oil	88.8 l
13 Sep 1999	Herbicide spray	Glyphosate	2.8 l
	Insecticide spray	Chlorpyrifos	0.47 l
23 Sep 1999	Mowing	—	—
2 Nov 1999	Fertilizer	Dolomite	2.47 Ton
		15-2-15	0.50 Ton
	Disking 1 way	—	—
29 Feb, 2000	Fertilizer	16-0-16	0.61 Ton
9 May 2000	Disking 1 way	—	—
30 May 2000	Herbicide spray	Glyphosate	2.80 l
	Insecticide spray	Chlorpyrifos	0.47 l
1 Jul 2000	Fertilizer	15-2-15	0.61 Ton
	Disking 1 way	—	—
17 Jul 2000	Insecticide spray	Petroleum oil	56.1 l
	Fertilizer	Techmangam	12.0 Kg
		Zinc	5.6 Kg
2 Nov 2000	Fertilizer	15-2-15	0.61 Ton
18 Nov 2000	Disking 1 way	—	—

Table 2.2. Two-way analysis of variance for data on numbers of phytoseiids found on the east, inner and west sides of the tree canopy at different sampling times on indicated dates during the year.

Date	Source	df	MS Effect	F value	P-value
14 Oct 1999	Side	2, 81	0.754	3.348	0.040
	Hours	8, 81	0.628	2.789	0.008
	Interaction	16, 81	0.376	1.671	0.069
20 Oct 1999	Side	2, 81	1.066	3.253	0.043
	Hours	8, 81	0.401	1.224	0.295
	Interaction	16, 81	0.286	0.875	0.598
28 Oct 1999	Side	2, 81	101.009	13.423	< 0.001
	Hours	8, 81	8.363	1.111	0.364
	Interaction	16, 81	6.1039	0.811	0.669
9 Mar 2000	Side	2, 54	38.616	34.216	< 0.001
	Hours	5, 54	1.502	1.330	0.265
	Interaction	10, 54	0.882	0.781	0.645
16 Mar 2000	Side	2, 54	99.328	55.948	< 0.001
	Hours	5, 54	2.799	1.576	0.182
	Interaction	10, 54	1.382	0.778	0.648
17 Aug 2000	Side	2, 54	38.616	34.216	< 0.001
	Hours	5, 54	1.502	1.330	0.265
	Interaction	10, 54	0.882	0.781	0.645
24 Aug 2000	Side	2, 54	99.328	55.948	< 0.001
	Hours	5, 54	2.799	1.576	0.182
	Interaction	10, 54	1.382	0.778	0.648

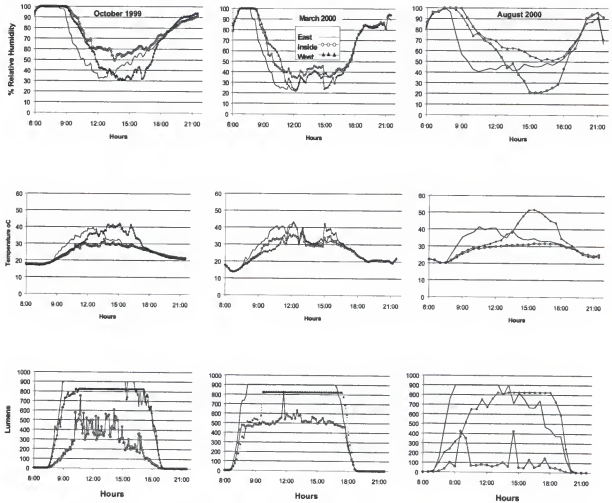


Figure 2.1. Daily average relative humidity (%), temperature ($^{\circ}\text{C}$) and light intensity (Lumens) on 14, 20 and 28 October 1999, 9 and 16 March 2000, and 17 and 24 August 2000 on the east, west and inside areas of the tree canopy at the Bader orchard in Highland City, Florida.

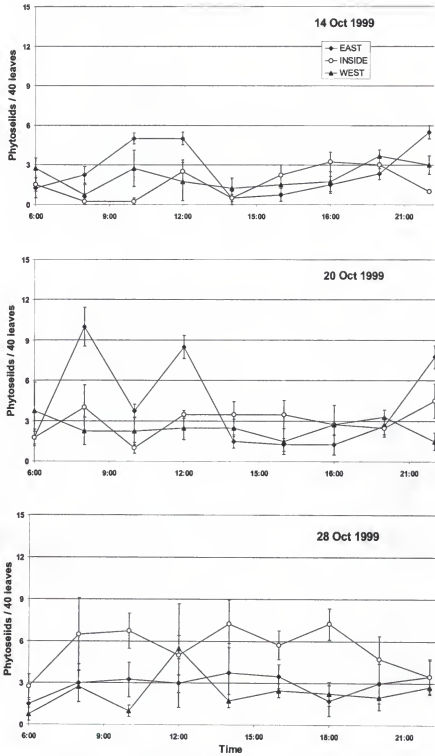


Figure 2.2. Mean number of phytoseiid motiles \pm SEM for 14, 20 and 28 October 1999. Values bearing the same letter were not significantly different using the HSD Tukey's test ($P < 0.05$).

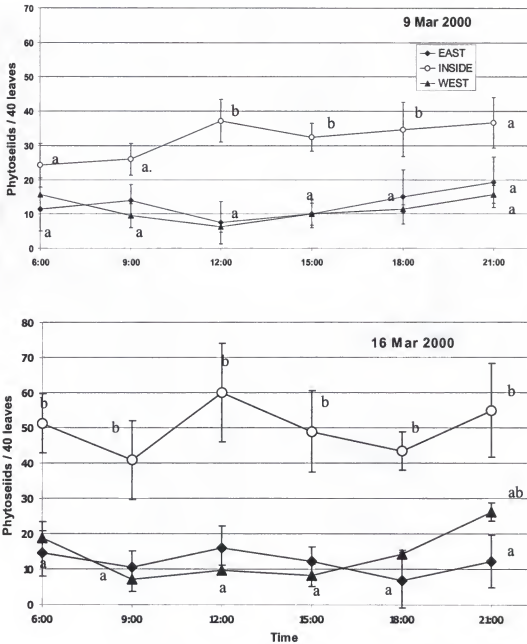


Figure 2.3. Mean number of phytoseiid motiles \pm SEM for 9 and 16 March 2000. Values bearing the same letter were not significantly different using the HSD Tukey's test ($P < 0.05$).

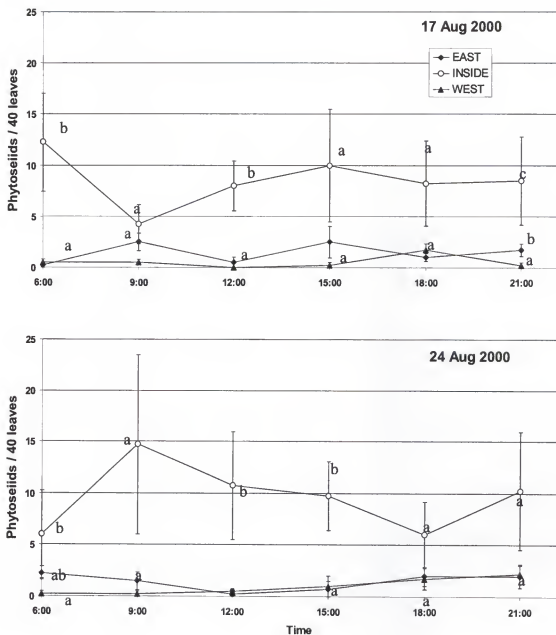


Figure 2.4. Mean number of phytoseiid motiles \pm SEM for 17 and 24 August 2000. Values bearing the same letter were not significantly different using the HSD Tukey's test ($P < 0.05$).

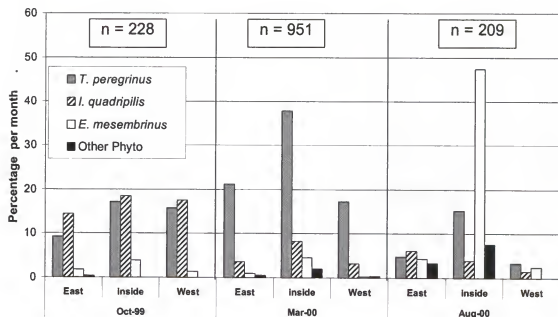


Figure 2.5. Percentage of phytoseiid mites identified from 30% of the total numbers found on October 1999, March and August 2000. Text box number indicates the total number of phytoseiid mites identified each month.

CHAPTER 3

LEAVES DAMAGED BY THE CITRUS LEAFMINER PROVIDE REFUGES FOR PREDATORY MITES IN CITRUS

Introduction

Topographical or morphological changes on the leaf surface due to feeding and/or damage by pests may produce changes in small arthropod populations including mites. One of the pests that causes change of leaf surfaces is the citrus leafminer (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). The CLM is a recently introduced pest into the USA. It was reported for the first time in Dade County, Florida in 1993 followed by rapid spread throughout the rest of Florida (Heppner 1993) and neighboring states including: Alabama, Louisiana and Texas in August 1994 (Legaspi et al. 1999). The CLM was initially considered a serious pest in Florida but is currently under control by native natural enemies (Amalin et al. 2001), introduced parasitoids (Hoy and Nguyen 1997) and IPM programs. Today, the CLM is considered a secondary pest problem (Browning et al. 2001). Adult CLM females deposit eggs singly on young citrus foliage (flush) preferring the midrib on the abaxial surface, and occasionally on green twigs and young fruits. Immediately after hatching, the CLM larva bores through the leaf epidermis and forms a mine due to the ingestion of the outer epidermal cells. Feeding by the larva leaves the outer epidermal wall and cuticle intact to form the outer wall of the mine (Sohi and Verma 1965, Achor et al. 1997). Later, chlorotic leaf patches are

observed where the larva mined. The mine is serpentine in form and each succeeding larval instar widens the tunnel. Mines caused by CLM larvae prevent young leaves from expanding uniformly with folding and/or twisting of leaves eventually occurring. The third larval instar causes most of the damage and this prepupal larva prepares a pupal chamber by folding the edge of the leaf down or upwards and binding it together with silk (Hoy and Nguyen, 1997). This damage facilitates other insects such as aphids and mealybugs to continue feeding on the leaf and the citrus plant becomes increasingly vulnerable to infection by Asiatic citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Hasse). Large amounts of inoculum are produced in these mined areas that promote the spread of the bacterium by rain splash (Gottwald et al. 1997).

Phylloplane fungi may have better growth in mined leaves than in healthy leaves (Timmer, personal communication). Phylloplane epiphytic fungi grow externally on a leaf surface without parasitizing it. During the winter months, these fungi can develop better in mined leaves than in healthy leaves due to the sheltered microenvironment provided by the damaged leaves. Most mined leaves maintain their photosynthetic capacity although it is reduced during the remainder of their development. Wang et al. (1999) identified 68 different isolate species of phylloplane epiphytic fungi on citrus in China.

Leaves folded at the edge by third instar CLM are sheltered spaces that provide refugia to other small arthropods. These spaces may provide more favorable microenvironment conditions than leaves without CLM damage. The sheltered spaces resemble structures called domatia that are present on leaves of many tropical and subtropical plants (O'Dowd and Wilson 1989). Domatia are located on the lower surface

of the leaves of many plants and occur in many different forms. Domatia are located at the junction of the primary and secondary veins and sometimes are protected by leaf hairs (O'Dowd and Willson 1997, Norton et al. 2001). Domatia are classified in one of the following types: pockets, cavities beneath expanded veins, pits, cavities which sink into the mesophyll, dense hair tufts; and rolled leaf margins, which are less common (Pemberton and Turner 1989)

The physiological functions of domatia are unknown but it is hypothesized that they have a co-evolutionary development with natural enemies, as a protective association against pests (O'Dowd and Willson 1989, Agrawal 1997, Agrawal and Karban 1997). Various authors have demonstrated that when these structural openings are covered or blocked the populations of predacious phytoseiid mites diminish, while pest mites increase. When artificial domatia were placed on leaves by attaching small pockets on lower leaf surface, phytoseiid mite numbers increased and pest numbers decreased (Walter 1996). A grapefruit leaf or any citrus leaf of commercial varieties is smooth and lacks domatia. In contrast, leaves with domatia often times have a rougher surface with veins protruding and/or hairs occurring around the axes of the veins.

Phytoseiid mites were classified into four categories by McMurtry and Croft (1997): Type I, specialized predators of *Tetranychus* species represented by *Phytoseiulus* species; Type II, selective predators of tetranychids mites represented by *Galendromus*, some *Neoseiulus* and a few *Typhlodromus* species; Type III generalist predators represented by most *Typhlodromus* and *Amblyseius* species; and Type IV specialized pollen feeders/generalist predators represented by *Euseius* species. The three most prevalent phytoseiids on Florida citrus are: *Euseius mesembrinus* (Dean), *Typhlodromalus*

peregrinus (Muma) (Childers 1994a) and *Iphiseiodes quadripilis* (Banks) (See Chapter 2 and Childers and Denmark unpublished). All three species completed their life cycles and reproduced on pollen (Abou-Setta 1987, Peña 1992, Badii and Hernandez 1993, McMurtry and Croft 1997, see chapter 4 for *I. quadripilis*). Similar studies have been shown with other phytoseiids in different parts of the world (Castagnoli et al. 1999, van Rijn and Tanigoshi 1999). It is apparent that many of the phytoseiids in Florida belong to either type III and IV species. Furthermore, one of the phytoseiid peaks in Florida coincides with citrus, *Quercus* sp. and *Pinus* sp. flowering and the species involved are: *E. mesembrinus*, *T. peregrinus* and *I. quadripilis* (Childers and Abou-Setta, 1999). *Quercus* sp. and *Pinus* sp. are commonly found around citrus orchards as wind breaks. Pollen grains from these plants and other species of weeds and shrubs accumulate on the adaxial surface of citrus leaves and almost certainly the three phytoseiids forage for pollen to complement their diet breadth. Studies between the density of pollen grains and phytoseiid abundance were completed in apple orchards in Canada with *Typhlodromus pyri* Scheuten (Addison et al. 2000), and on citrus in South Africa with *Euseius addoensis addoensis* (van der Merwe and Ryke) (Grout and Richards 1992a, b). These studies demonstrated a high correlation between early pollen availability and phytoseiid abundance in the field. For convenience and considering that this study was developed during the time of the year with the highest pollen abundance (*Citrus* sp., *Quercus* sp. and *Pinus* sp. flowering occurs by the end of February to middle March); the relationship between pollen grains deposited on the adaxial leaf surface and phytoseiid abundance on leaves was studied.

Two main objectives are pursued in this chapter. First, to compare the relative abundance of phytophagous and predatory mite populations between CLM damaged leaves, mature healthy leaves and young leaves. Second, the relationship between numbers of phytoseiids and pollen grains found on sampled leaves in the same orchard for the duration of this study was assessed.

Material and Methods

Weekly samples were taken from eleven-year-old 'Ruby red' grapefruit trees at the Citrus Research and Education Center in Lake Alfred. All ten sample trees were in one row and selected at random. Samples were taken from 2 February to 13 April 2001 between 1000 and 1100 hours and consisted of terminals with 5 leaves. Terminals were of three types: healthy mature terminals without CLM damage and terminals with leaves that had suffered CLM damage and young terminals with 5 leaves. The first two types of terminals had leaves that flushed the previous year while the third type of terminals were sampled from 23 March through 13 April. These latter terminals started to flush around mid-March 2001. This grove had not received any pesticide sprays since September of the previous year. On 5 April, the grove was sprayed with carbaryl (Sevin® 4F) at a rate of 94.1 kg/ha in 2.8 Kl of water to control the eastern lubber grasshopper *Romalea microptera* (Beauvois) (= *R. guttata*) (Orthoptera: Acrididae). The spray was applied using a handgun directed to the ground and up to 1 m high on the trees because the flightless grasshopper nymphs concentrated there.

Pollen counts were taken from 2 February to 16 March (during grapefruit flowering in this orchard). One healthy leaf from each of the same 10 trees sampled was collected for pollen counts. In the laboratory, immediately after sampling, a 5 to 7 cm long transparent adhesive tape was placed along the middle vein on the upper leaf surface of each leaf and

left there for 3 to 5 minutes, then removed, placed in a wax paper, labeled and stored in the refrigerator until processed. Slides were prepared from 1 cm² sample areas of the adhesive tape. These 1 cm² areas of tape were placed individually on the slide with the adhesive part up and then a drop of dye was added followed by placement of the cover slide (Addison et al. 2000). The dye was prepared with 0.2 g of Trypan Blue in 200 ml of 50% glycerol. This dye is used for ascospore staining (Skaria and Tao 1996) and glycerol will cause pollen grains to slightly swell (Addison et al. 2000). A contrast microscope was used in the laboratory to count pollen grains, with a magnification of 400X. The area for counting pollen grains (field of view) was approximately 1.130 mm². All pollen grains in this field of view were counted and the area within the field of view was calculated with a Hemacytometer Bright Line ® (Reichert, Buffalo, NY). Pollen counts were completed as follows. The first grain of pollen found searching the slide was centered in the middle of the field of view and then all the pollen grains in that view were counted.

The three types of terminals were taken to the laboratory and counts of phytophagous and predatory mite eggs and motiles were taken using a stereomicroscope. Data were transformed using $\sqrt{X+0.5}$, where X is the number of eggs or motile mites per 5-leaf terminals. ANOVA was used to analyze the data and the means for the last three sample dates were compared using an LSD test (Multiple Range Test for separation of means using Statistica ® (Stat Soft, Inc. 2000). Means \pm SEM values shown here are untransformed data. Pollen counts and the total number of phytoseiids present from leaf samples on the indicated dates were evaluated to determine correlations between the two factors.

Results

Pests mites found in this study included: *Eutetranychus banksi* (TCM), *Eotetranychus sexmaculatus* (6-SSM), citrus rust mites (CRM and PRM) and a few *Brevipalpus* sp., while not a single *Panonychus citri* was found. The phytoseiids: *E. mesembrinus*, *T. peregrinus* and *I. quadripilis*; the stigmaeid *Agistemus* sp., and a few *Stethorus* sp. (Coccinellidae) larvae and adults were also found. *Stethorus* sp. is a predator associated with *E. sexmaculatus* (Muma et al. 1961). Tydeids mites were also present in this orchard, but their populations were low with no significant differences found among the three types of terminals ($P > 0.05$).

Tables 3.1 and 3.2 present the ANOVA and the least significant difference (LSD) tests for *E. banksi* eggs and motiles, respectively. Figures 3.1 and 3.2 present the means (\pm SEM) for *E. banksi* eggs and motiles, respectively. Significant differences ($P < 0.05$) were found in the numbers of *E. banksi* eggs on 23 March and 13 April, when *E. banksi* preferred to oviposit on the new young leaves (Fig. 3.1). However, no differences were found for *E. banksi* motiles on any of the dates (Fig. 3.2).

Tables 3.3 and 3.4 present the ANOVA and the LSD tests for *E. sexmaculatus* eggs and motiles, respectively. Figures 3.3 and 3.4 present the means (\pm SEM) for *E. sexmaculatus* eggs and motiles, respectively. Significantly ($P < 0.01$) higher numbers of both *E. sexmaculatus* eggs and motiles were found on mined leaves than on healthy leaves. Significant differences in mean numbers of *E. sexmaculatus* eggs were found on 2, 8, 23 and 31 March and 13 April (Fig. 3.3). Significantly higher numbers of *E. sexmaculatus* motiles were obtained on leafminer leaves than on healthy mature and young leaves on 23 February; 2, 8, 23 and 31 March; and 7 and 13 April (Fig. 3.4).

No significant differences ($P > 0.05$) were found for citrus rust mites on any of the dates sampled (Table 3.5 and Fig. 3.5). During this period, the peak citrus rust mite population occurred in mid February (c.a. 100 RM / 5 leaves on healthy mature leaves and 75 RM / 5 leaves on leafminer damaged leaves). This population decreased to its lowest levels on 9 April (< 5 RM / 5 leaves on all three types of leaves). Phytoseiid numbers increased from 8 February until the end of March with the largest numbers of phytoseiids occurring on mined leaves ($P < 0.05$). Significantly higher numbers of phytoseiids were found on 2 February, 2, 8, 16, 23 and 31 March, and 7 April (Fig. 3.6). The highest numbers of phytoseiids occurred on 23 March with 8.9 ± 2.0 (mean \pm SEM) motiles / 5 leaves on leafminer damaged leaves, whereas for healthy mature leaves there were 1.9 ± 0.6 phytoseiid motiles / 5 leaves. Table 3.7 and Fig. 3.7 present the ANOVA and the LSD tests, and means (\pm SEM) for motile stigmatheids. Although high numbers of stigmatheid predators were found on mined leaves they were not significantly different due to the patchy distribution of these mites with one exception on 23 February (Fig. 3.7). Permanent slides were prepared and identifications were made with most of the phytoseiids found on 16, 23 and 31 March. They were identified as *Iphiseiodes quadripilis* ($n = 139$), *Typhlodromalus peregrinus* ($n = 122$), *Euseius mesembrius* ($n = 18$), and 1 each of *Phytoscutus sexpilis* and *Galendromus helveolus* (Fig. 3.8).

Pollens from different types of plants were found on the grapefruit leaves, including *Quercus* sp., *Pinus* sp., citrus, and unrecognized pollen types. There were no differences in the types of pollen found but the total number of pollen grains found on the field of view increased as the plants started flowering. Grapefruit trees in this orchard bloomed between 2 March and 16 March with most of the flowers already shed by 16 March. The

correlation between the number of pollen grains in the field of view and phytoseiid mites was positive and highly significant ($P = 0.004$) using the Pearson correlation coefficient with a $R^2 = 0.83$ (Fig. 3.9) (Statistica, 2000). Temperatures ($^{\circ}\text{C}$) and rainfall (cm) records for the duration of this study are presented in Figure 3.9.

Discussion

Numbers of phytophagous mites varied on the three types of leaves sampled. No preference for leaf type was shown by *E. banksi*, but *E. sexmaculatus* preferred mined leaves. *E. banksi* produce little webbing compared to *E. sexmaculatus*, and as expected, they should have exploited the healthy mature or young leaves. However, on the 23 March and 13 April samples, the numbers of oviposited *E. banksi* eggs were higher on young flushes than on healthy mature or leafminer damaged leaves. Henderson and Holloway (1942) reported a similar preference of oviposition on new leaves by *P. citri*. The reason for this was that younger leaves might be more nutritious than older leaves. The younger leaves were also expanding when carbaryl was sprayed on 5 April. The pesticide might not have contacted most of these younger leaves on the sample of 13 April thus new expanding leaves could be used by *E. banksi* to oviposit eggs on leaves less affected by the pesticide toxicity. It has been reported that carbaryl has little effect on tetranychids but on occasions can stimulate population increases (Dittrich et al. 1974). It is possible that carbaryl has an apparent short negative effect on *E. banksi* populations because the number of eggs and motiles decreased after the spray (Fig. 3.1 and 3.2) and similarly the physical effect of the hydraulic spray by itself has resulted in temporary reduction of *E. banksi*. Also, it is likely that the high numbers of *E. sexmaculatus* eggs were the result of a stimulatory effect by carbaryl.

Although *E. sexmaculatus* is seasonally more abundant following cold winter months and prefers older, harder leaves (Childers, 1994a), the mean numbers of *E. sexmaculatus* eggs and motiles were significantly more abundant on mined leaves than on healthy mature leaves (Figs. 3.3 and 3.4). As expected, the mined leaves provided increased spaces for webbing for this mite. An important feature here is that we observed the sheltered effect of the mined leaves on *E. sexmaculatus*. *Eotetranychus sexmaculatus* had a very moderate decrease in its population after carbaryl was applied but immediately increased by the following sample date (13 April). This increase may not only be due to the protective affect of the mines but also to a hormoligosis effect by the pesticide as explained for many other tetranychid pests. Hormoligosis is described as stimulation by small quantities of a stressor that enhances fertility (Dittrich 1987). Dittrich et al. (1974) demonstrated that the Female:Male ratio on *Tetranychus urticae* Koch increased significantly after these mites were left on leaves treated with carbaryl.

Phytoseiids and stigmatheids were found in higher numbers on leafminer damaged leaves than on healthy mature and younger leaves (Figs 3.6 and 3.7). Mean numbers of phytoseiids were significantly ($P < 0.05$) different, with numbers ranging from 2 to 5 times greater on leafminer damaged leaves versus healthy mature leaves. Mined leaves not only provided protective spaces for mites but also more prey and other types of food. (Villanueva and Harmsen 1996, Villanueva 1997) observed similar effects on mines produced by the spotted tentiform leafminer *Phyllonorycter blancardella* Fabricius (Gracillariidae) on apple in Ontario. The type of mines formed on apple was different from CLM mines. Both stigmatheids (*Zetzellia mali* (Ewing)) and phytoseiids (*Typhlodromus caudiglans* Schuster and *Neoseiulus fallacis* (Garman)) were found on old

mines on apple feeding on *Panonychus ulmi* and tarsonemids (Villanueva and Harmsen 1996, 1998). The tarsonemids oviposited higher numbers of eggs around the frass and exuviae inside the mines on apple. Frass and exuviae are also found in mines produced by CLM and unpublished observations by Villanueva (unpublished) in the laboratory showed that *I. quadripilis* and *E. mesembrinus* feed on phylloplane fungi that grow more abundantly on mined leaves than on healthy leaves.

Here phylloplane is used in reference to a leaf as a habitat for microorganisms. More importantly, epiphytic fungi had recently been studied for their antagonistic effects to pathogenic fungal diseases such sooty mold on mandarin (Srivastava and Thakre 2000) and citrus canker on lemon (Pabitra et al. 1996). Theoretical approaches to the use of entomopathogens as "body guards" of plants have been formulated and discussed recently (Elliot et al. 2000). Hargreaves and Parappukkaran (2000) noted that most common fungi occurring outdoors are those that live on the surfaces of leaves (phylloplane fungi). A few genera, *Cladosporium*, *Alternaria*, *Epicoccum* and *Aureobasidium* comprise between 40% and 80% of the propagules in the atmosphere in surveys worldwide. Fatch et al. (1995) noted that species of epiphytic fungi varied depending on season, age of leaves and plant variety on grape. In the acarine system, Belczewski and Harmsen (1997) found that sprays of the phylloplane fungus *Alternaria alternata* Keissler on apple leaves in the field enhanced population growth of *Tetranychus urticae* compared to that of *Panonychus ulmi*. Whereas Zemec and Prenerova (1997) observed that *T. pyri* fed on the conidia of powdery mildew species *Erysiphe orontii* Castellani from tobacco and *Oidium fragariae* Harz from strawberry in the laboratory. *Typhlodromus pyri* reproduced and completed its life cycle on *E. orontii*.

The relative abundance of the phytoseiids *I. quadripilis* and *T. peregrinus* is shown in Fig. 3.8 on the three types of leaves sampled on 16, 23 and 31 March. These three dates had the highest phytoseiid numbers. *Iphiseiodes quadripilis* and *T. peregrinus* combined for more than 90% of the total number of phytoseiids found in this study. This coincides with Childers and Denmark survey (unpublished) where *T. peregrinus* and *I. quadripilis* are the second and third most abundant phytoseiids in seven central Florida orchards. However, *E. mesembrinus* was the most abundant phytoseiid in their survey but was not abundant in this study. *Iphiseiodes quadripilis* and *T. peregrinus* are generalist predacious mites (Muma 1961) and the former shows preference for grapefruit over orange (see chapter 5). The biology and behavior of *T. peregrinus* have been studied more than *I. quadripilis*. *Typhlodromus peregrinus* fed on pollen, *P. latus*, *P. oleivora* and tetranychids (Peña 1989). It may be reasonable to think that *E. mesembrinus* was not abundant due to its phenology and may be less fit for the exploitation of mined leaves than *I. quadripilis* and *T. peregrinus*. Stigmaeids were found regularly during the duration of this study, but their distribution was localized. Hence variability is observed in the high SEM values obtained (Fig. 3.7). Similar observations in patchy distribution of *A. floridanus* were made by Muma and Selhime (1971).

Both phytoseiid numbers and pollen grains increased between 2 February and 16 March. Similar observations are reported on pollen abundance and increases of phytoseiid populations in many crops. Addison et al. (2000) suggested that *T. pyri* abundance had a positive correlation with early season pollen density than with the abundance of the mite prey (*Aculus schlechtendali* Nalepa) in apples. Similarly, when *Euseius tularensis* Congdon was released into navel orange groves, the phytoseiid

exhibited a significantly greater increase in groves with a ground cover crop of mixed leguminous plants over a 4-week period than in groves without ground cover crop in California (Grafton-Cardwell et al. 1999). The results shown here had similar responses and the high correlation coefficient obtained ($r = 0.91$) may explain *I. quadripilis* and *T. peregrinus* abundance during citrus flowering (Fig. 3.9).

This chapter describes two ecological components that affect phytoseiid populations on citrus: the mine caused by *P. citrella* and pollen deposited on the leaves. The mine provides phytoseiids shelter and food. Phytoseiids utilize mines as refugia where they can increase more than on healthy leaves due to better micro-environmental conditions. The phylloplane fungi developing within the mines and the leafminer frass are also utilized as food sources. This can be important especially during the winter months when prey availability is scarce. Pollen is a proven food source of phytoseiids and pollens of *Quercus* sp, *Pinus* sp. and *Citrus* sp. coincided with increased phytoseiid numbers in the field. Both *I. quadripilis* and *T. peregrinus* are generalist phytoseiids mites that complete their life cycles and reproduced on pollen diets in the laboratory (Muma 1967b and 1971, Peña 1992, see Chapter 4). The citrus leafminer, *P. citrella* is not considered a beneficial insect but changes in leaf morphology produced by this pest are used positively by phytoseiids and thus augment pest mite control. A practical application of this research is that sampling on mined leaves alone will provide increased phytoseiid numbers. However, it also may be possible that only one or more predatory species such as *I. quadripilis* and *T. peregrinus* exploit this type of habitat while other phytoseiid species avoid it. This study only focused on acarine populations but insects such thrips, psyllids, curculionids, hemipterans and arachnids were observed more frequently on mined leaves

than on healthy mature leaves. More research is needed because the mines can affect beneficial arthropod numbers not only on citrus but in other plants too.

Table 3.1. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of *Eutetranychus banksi* eggs on healthy mature, leafminer-damaged and young leaves.

Date	df	F value	P	LSD test
02-FEB	1, 18			-
08-Feb	1, 18	1.44	0.24	-
16-Feb	1, 18	0.35	0.56	-
23-Feb	1, 18	0	1	-
02-Mar	1, 18	0.16	0.69	-
08-Mar	1, 18	0.26	0.61	-
16-Mar	1, 18	0.21	0.64	-
23-Mar	2, 27	4.95	0.01	*
31-Mar	2, 27	2.10	0.14	n.s.
07-Apr	2, 27	4.41	0.02	*
13-Apr	2, 27	5.23	0.01	**.

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

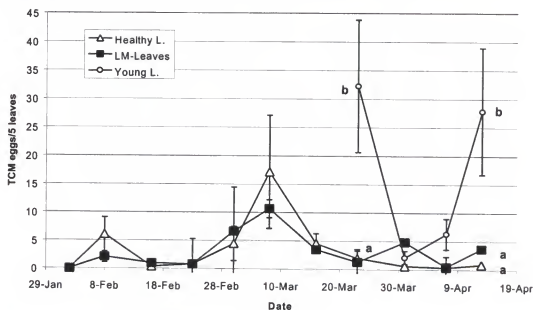


Figure 3.1. Mean numbers (\pm SEM) of *Eutetranychus banksi* eggs on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.2. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of *Eutetranychus banksi* motiles on healthy mature, leafminer-damaged and young leaves.

Date	d.f.	F value	P	LSD test
02-Feb	1, 18			-
08-Feb	1, 18	0.19	0.66	
16-Feb	1, 18	1.20	0.28	
23-Feb	1, 18	0.91	0.35	
02-Mar	1, 18	0.02	0.86	
08-Mar	1, 18	0.003	0.95	
16-Mar	1, 18	0.12	0.72	
23-Mar	2, 27	1.63	0.21	n.s.
31-Mar	2, 27	2.15	0.13	n.s.
07-Apr	2, 27	0.53	0.58	n.s.
13-Apr	2, 27	1.57	0.22	n.s.

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

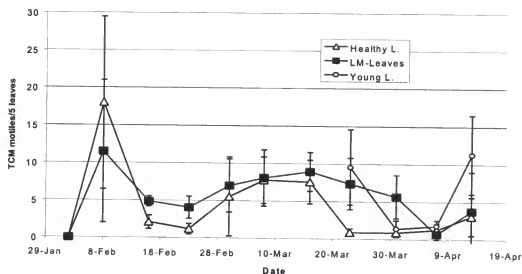


Figure 3.2. Mean numbers (\pm SEM) of *Eutetranychus banksi* motiles on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.3. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of *Eotetranychus sexmaculatus* eggs on healthy mature, leafminer-damaged and young leaves.

Date	d.f.	F value	P	LSD test
02-Feb	1, 18			-
08-Feb	1, 18	1.21	0.28	
16-Feb	1, 18	1.40	0.25	
23-Feb	1, 18	4.7	0.04	
02-Mar	1, 18	5.63	0.02	
08-Mar	1, 18	9.27	0.006	
16-Mar	1, 18	0.01	0.92	
23-Mar	2, 27	5.53	0.009	**
31-Mar	2, 27	6.78	0.004	**
07-Apr	2, 27	2.87	0.07	n.s.
13-Apr	2, 27	7.68	0.002	**

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

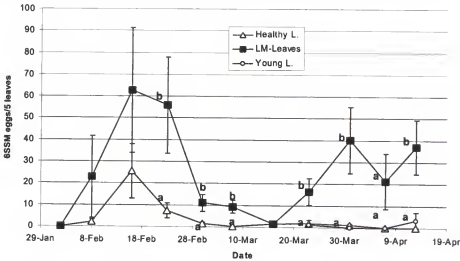


Figure 3.3. Mean numbers (\pm SEM) of *Eotetranychus sexmaculatus* eggs on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.4. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of *Eotetranychus sexmaculatus* motiles on healthy, leafminer-damaged and young leaves.

Date	d.f.	F value	P	LSD test
02-Feb	1, 18	0.79	0.38	-
08-Feb	1, 18	0.90	0.35	
16-Feb	1, 18	1.53	0.23	
23-Feb	1, 18	5.58	0.02	
02-Mar	1, 18	4.83	0.04	
08-Mar	1, 18	7.05	0.01	
16-Mar	1, 18	0.54	0.46	
23-Mar	2, 27	6.12	0.006	**
31-Mar	2, 27	8.93	< 0.001	**
07-Apr	2, 27	8.44	< 0.001	**
13-Apr	2, 27	7.65	0.002	**

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

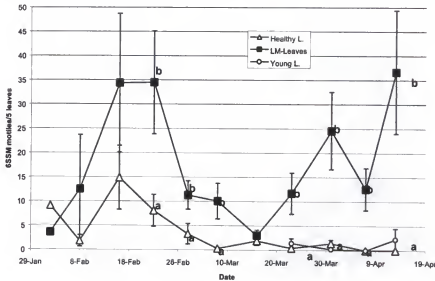


Figure 3.4. Mean numbers (\pm SEM) of *Eotetranychus sexmaculatus* motiles on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.5. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of citrus rust mite motiles on healthy, leafminer-damaged and young leaves.

Date	d.f.	F value	P	LSD test
02-Feb	1, 18			-
08-Feb	1, 18	0.167	0.68	
16-Feb	1, 18	3.35	0.08	
23-Feb	1, 18	0.01	0.90	
02-Mar	1, 18	0.86	0.36	
08-Mar	1, 18	0.133	0.71	
16-Mar	1, 18	2.52	0.12	
23-Mar	2, 27	2.34	0.11	n.s.
31-Mar	2, 27	1.32	0.28	n.s.
07-Apr	2, 27	1.06	0.35	n.s.
13-Apr	2, 27	1.39	0.26	n.s.

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

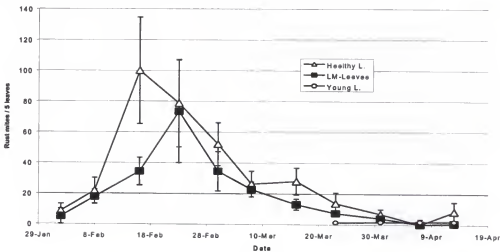


Figure 3.5. Mean numbers (\pm SEM) of citrus rust mite motiles on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.6. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of phytoseiid mite motiles on healthy, leafminer-damaged and young leaves.

Date	d.f.	F value	P	LSD test
02-Feb	1, 18	5	0.03	-
08-Feb	1, 18	0	1	-
16-Feb	1, 18	1.14	0.29	-
23-Feb	1, 18	3.09	0.09	-
02-Mar	1, 18	5.26	0.03	-
08-Mar	1, 18	4.47	0.04	-
16-Mar	1, 18	28.78	< 0.01	-
23-Mar	2, 27	14.91	< 0.01	**
31-Mar	2, 27	17.32	< 0.01	**
07-Apr	2, 27	3.84	0.03	*
13-Apr	2, 27	2.39	0.11	n.s.

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$) on the LSD test.

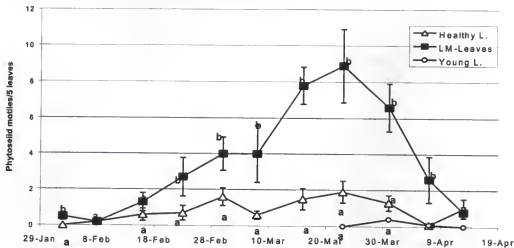


Figure 3.6. Mean numbers (\pm SEM) of phytoseiid motiles on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

Table 3.7. Analysis of variance (first seven rows) and least significant difference (LSD) analysis (last four rows) for mean numbers of stigmæid motiles on healthy, leaf miner-damaged and young leaves.

Date	d.f.	MSE	F value	P	LSD test
02-Feb	N/A	N/A	N/A	N/A	-
08-Feb	1, 18	16.2	0.89	0.35	-
16-Feb	1, 18	3.2	3.64	0.07	-
23-Feb	1, 18	57.8	5.41	0.03	-
02-Mar	1, 18	80	1.26	0.27	-
08-Mar	1, 18	64.8	3.38	0.08	-
16-Mar	1, 18	28.8	3.62	0.07	-
23-Mar	2, 27	1.48	2.43	0.10	n.s.
31-Mar	2, 27	0.79	1.17	0.32	n.s.
07-Apr	2, 27	0.19	2.20	0.12	n.s.
13-Apr	2, 27	0.42	1.65	0.20	n.s.

One asterisk (*) means that there are significant differences ($P < 0.05$) and two asterisks means that differences are highly significant ($P < 0.01$).

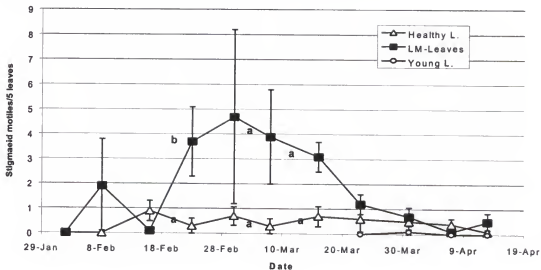


Figure 3.7. Mean numbers (\pm SEM) of stigmæid motiles on healthy mature, leafminer-damaged and young leaves. Values bearing the same letter were not significantly different in an ANOVA or LSD test ($P > 0.05$) on a given sampling date.

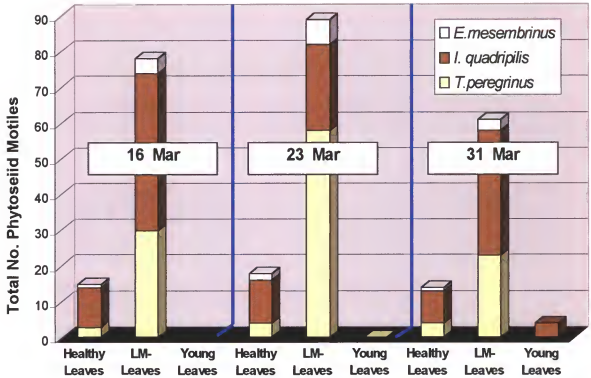


Figure 3.8. Relative abundance of *Euseius mesembrius*, *Iphiseiodes quadripilis* and *Typhlodromus. peregrinus* on healthy mature, leafminer damaged leaves by *Phyllocnistis citrella* and young grapefruit leaves on three dates with the highest numbers of phytoseiids registered.

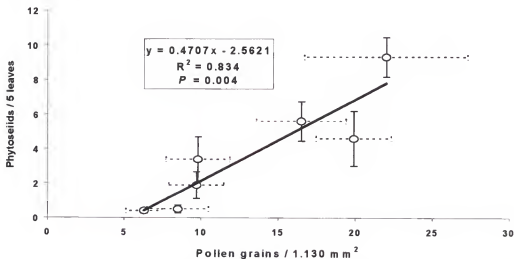


Figure 3.9. Relationship between phytoseiids (\pm SEM) and pollen grains (\pm SEM) found on grapefruit leaves from 2 February to 16 March 2001.

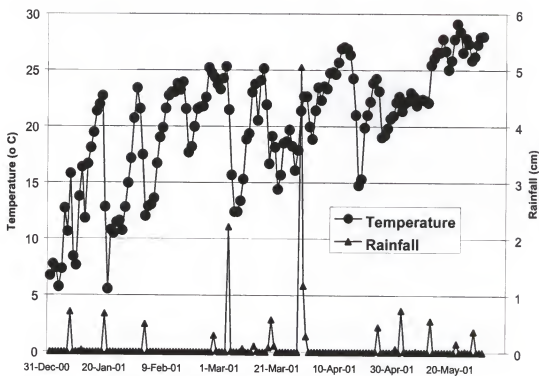


Figure 3.10. Temperature and rainfall recorded on the Florida automated weather network CREC-Lake Alfred Station from 31 December 2000 to 20 May 2001.

CHAPTER 4
COMPARATIVE DEVELOPMENT OF *IPHISEIODES QUADRIPILIS* (BANKS)
(ACARI: PHYTOSEIIDAE) ON VARIOUS NATURAL DIETS AND PREDATION
ON *ACULOOPS PELEKASSI* (KEIFER) (ACARI: ERIOPHYIDAE) IN THE
LABORATORY

Introduction

Phytoseiid mites are considered effective biological control agents on numerous crop systems (Baillod *et al.* 1982; Bostanian and Coulombe 1986; Brodsgaard and Hansen 1992; De Moraes and McMurtry 1985; and Yaninek *et al.* 1998). They prey on phytophagous mites in Florida citrus orchards but their potential use in controlling mite pests in orchards under IPM regimes is not well known. Muma (1975) found 19 species of phytoseiids associated with citrus trees but this number is probably low. One of the more abundant phytoseiid mites on Florida citrus is the Shiny button mite (SBM) *Iphiseiodes quadripilis* (Banks) and it is considered the third most abundant phytoseiid today (Childers and Denmark, unpublished data). The SBM has been described as a general facultative predator that can feed on eriophyids, tetranychids and honey + citrus pollen and other lesser economically important citrus mites (Muma 1971). However, little is known about the life cycle of SBM, its predatory behavior, or the ecological factors that influence its development. The SBM can be easily observed visually in the field by the naked eye and it is distinguishable from most other phytoseiid mites by its shiny, dark, mahogany-red color. The body shape of adults is almost round and swollen to a nearly hemispheric profile (Muma 1975).

The citrus rust mite (CRM) *Phyllocoptruta oleivora* (Ashmead) (Eriophyidae) is one of the key pests on Florida citrus and is found in all citrus-growing areas of the world. The CRM can cause considerable economic losses in the citrus industry due to direct feeding damage on green twigs, leaves and fruits and the high cost of acaricides for its control. Heavy infestations produce fruit russetting that decreases fruit quality (Childers 1994a). Pink citrus rust mite (PRM) *Aculops pelekassi* (Keifer) is another eriophyid found in Florida and is generally misidentified with CRM due to their small size. Although CRM has a yellowish color that is different from the pinkish coloration of PRM, this is distinguishable only at higher magnifications using a dissecting stereomicroscope. Even then coloration is not a clear indicator for species recognition and reliable identification requires slide mounting of specimens (Childers and Achor 1999).

Tetranychidae are common pests on citrus and the two most common species are the Texas citrus mite (TCM) *Eutetranychus banksi* (McGregor) and citrus red mite (CReM) *Panonychus citri* (McGregor) (Childers *et al.* 1991, Childers 1994a). These mites prefer dry conditions and are common in citrus areas in Texas and California, respectively. They are important pests in Florida from March through June or during other extended dry periods throughout the year (Childers 1994a). Both mites cause stippling on the leaves, mesophyll collapse at high densities and can feed on green twigs and fruits (Albrigo *et al.* 1981). On occasion, leaf drop in the upper portions of trees is associated with TCM feeding injury following dry weather conditions in Texas (Dean 1980).

Pollen can be an important alternative or supplementary component of diet for many predacious mites in the family Phytoseiidae (Abou-Setta and Childers 1987, Badii and

Hernandez 1993, Fouly et al. 1995, McMurtry and Croft 1997, Yamamoto 1994). Pollen is found in Florida throughout the year from wild flowers, various annual and perennial flowering plants, shrubs and ground cover plants and weeds. However, the most abundant period of tree pollen release occurs from late February to May when *Quercus* spp. (oak) (Fagaceae), *Pinus elliottii* Engelman, *P. palustris* Miller (Pinaceae), *Casuarina equisetifolia* J.R. Forsts and G. Forst, *C. cunninghamiana* Miquel (Casuarinaceae), *Taxodium distichum* (L.) (Taxodiaceae), *Juniperus silicicola* (Small) Bailey (Cupressaceae), *Myrica cerifera* L. (Miricaceae), *Broussonetia papyrifera* (L.) Vent (Moraceae), and *Morus rubra* L. (Moraceae) are blooming (Bucholtz et al. 1991). Citrus trees also bloom during this period. Oak trees produce profuse amounts of pollen during flowering and oak pollen is dispersed by wind and deposited on citrus leaves during this period. Alternatively, pollen of ice plant (*Malephora crocea* Jacquin, family: Aizoaceae) is often used to maintain phytoseiids in laboratory colonies and is sold commercially. Pollen has not been evaluated as a dietary component for SBM. It is not known which life stages feed on pollen or complete development and oviposition with a diet of pollen alone.

The objective of this study was to determine if *I. quadripilis* can complete its life cycle and oviposit successfully on diets of CRM, CReM, TCM and oak or ice plant pollens in the laboratory. Also, the acceptability of CRM and PRM as food sources to different *I. quadripilis* life stages was assessed.

Materials and Methods

Assays with natural diets

Reproductive SBM females were collected in the field and placed on leaf arenas in the laboratory with eggs and motile stages of CReM and ice plant pollen as food sources. SBM eggs were collected and placed individually onto arenas containing the different diets. Three species of prey mites were used in this experiment: CRM, CReM and TCM, and two types of pollen: ice plant and oak, and a separate treatment of water alone was included. Three types of arenas were employed. The first type was used to test SBM development without food and consisted of a square piece of ceramic tile approximately 5.0 to 7.0 cm in length per side, 1.0 cm thick and surrounded by a wet strip of cotton. The piece of tile was placed in a 15.0 cm diam. x 2.5 cm deep Petri dish filled with water to avoid mite escape and to maintain the desired moisture level on the cotton strips. The tile was beige in color, had a glazed-matte finish, and this surface was used for the arena. Each tile was divided into 16 squares (arenas) using thin wet cotton strips. SBM mites were introduced individually using a 5-0' sable brush.

The second type of arena (Fig. 4.1) was used to test the diet of SBM on CRM only and was prepared on an orange fruit c.a. 4.0 to 6.0 cm diam. Each fruit was placed on a PVC pipe (3.5 cm diam. x 1.5 cm tall) and then placed in a Petri dish (8.5 cm diam. by 1.5 cm deep) half filled with water to maintain moisture on the cotton strips during the duration of the experiment. Each fruit had a circular experimental area approximately 3.5 to 4.0 cm diameter on the upper part of the fruit that was surrounded with a 1.5 cm wide wet cotton strip to confine the mites. The orange fruits were chosen for the high numbers of eriophyid mites each contained (> 200 mites /cm²). All insects and mites other than the

citrus rust mites were removed. Most of the eriophyids in each arena were CRM based on the yellowish color of their bodies. One arena of this type was used for each SBM egg.

The third type of arena contained a grapefruit leaf placed in a 15.0 cm diam. x 2.5 cm deep plastic Petri dish. The leaf was placed with its upper surface facing up on the top of a wet foam pad. This arena was used to test the diet of CReM, TCM, or the two types of pollen. Wet cotton strips completely surrounded each leaf to confine the mites. Leaves used to prepare these arenas were cleaned with a solution of 5% sodium hypochlorite (bleach) to remove contaminants. The leaf arenas for the diets using CReM were divided into quarters using wet cotton strips. Within each arena, five female CReM were left for 3 days to lay eggs before placement of the SBM eggs per arena. Similar steps were made for the TCM diet. Arenas for the pollen diet were divided similarly and pollen was added at the same time with one SBM egg. More female CReM, TCM, or one of the two types of pollen were added to each respective arena to maintain development of the SBM during the duration of the experiment. The leaf arenas were replaced periodically before they started to deteriorate and leaves used for this purpose followed similar cleaning and preparation steps as described above.

The mean developmental times of the different SBM stadia (egg, larva, nymphs) and the preoviposition interval were analyzed using ANOVA followed by the LSD test (post hoc comparisons) and % survival to adults was analyzed using the test of difference between proportions (@Statistica 2000).

Predation of SBM deutonymphs and females on pink rust mite (PRM)

‘Valencia’ orange fruits approximately 4.0 to 6.0 cm diam. were brought to the laboratory and arenas (ca. 3.5 to 4.0 cm diameter) were prepared within areas containing

the highest concentrations of eriophyids found. This arena was similar to the type 2 unit described in the previous assay and was maintained with adequate moisture for 5 to 7 days. Mite species and insects were removed from the experimental arena with only the eriophyids remaining. Pink and yellow colored eriophyids were observed in the arenas, and samples of each were taken for identification. Identification made through permanent slides and electron microscopy photography showed that these mites were PRM and CRM, respectively (Fig 4.2).

The mean numbers of CRM and PRM were 66.2 ± 5.63 and 40.4 ± 1.57 per cm^2 , respectively, on the apex the arena. Most of the eriophyids were concentrated in areas nearer to the cotton strips. All arenas contained both PRM and CRM in excess. SBM deutonymphs reared with pollen and CReM in the laboratory were starved for 6 h and SBM adult females were starved for 6 and 24 h. After these starvation periods, the respective stages were transferred individually onto the arenas using a fine N° 5-0 sable brush followed by four-minute continuous periods of observation. The number of encounters and consumed eriophyids were recorded during this time. An encounter is defined as the change in the searching behavior of the SBM such as palpation and/or arrestment. The four-minute interval was determined because at this time 100 percent of the SBM females ($n=10$ per interval time) placed in the arena consumed 2 or more eriophyids. This time seemed to be appropriate to evaluate and compare eriophyid predation (Fig. 4.3). Comparisons of the means of eriophyids consumed and encountered by SBM deutonymphs and females, respectively, were assessed using the paired *t*-test (Excel ® 2000).

Results

Assays with natural diets

The SBM larval stage developed successfully to the protonymphal stage using CRM, CreM, TCM, ice plant or oak pollen diets and there were no significant differences among the diets in this stage ($P > 0.05$) (Fig. 4.4). However, on water alone and with the CRM only diet, all the SBM protonymphs stopped development and died. Survival of SBM to adults was obtained with oak pollen, ice plant pollen, TCM and CreM and percentage survival rates were of 68, 60, 48, 36 %, respectively. A significant difference in percentage survival of SBM to adults was obtained between the oak pollen alone and CreM alone diets ($p < 0.05$). The female:male ratios were 5:4 (CreM, 1 female died before laying an egg), 7:5 (TCM, 1 female died before laying an egg), 8:9 (oak, 1 female died before laying egg) and 8:7 (ice plant, 2 females died before laying eggs). SBM females completed their life cycles on CreM, ice plant pollen alone, oak pollen alone and TCM diets in 9.48 ± 0.2 ($n = 4$), 9.44 ± 0.5 ($n = 6$), 9.39 ± 0.3 ($n = 7$), 8.5 ± 0.2 ($n = 6$) days \pm SE, respectively ((Fig. 4.4).

Predation of SBM deutonymphs and females on PRM

SBM deutonymphs encountered significantly higher mean numbers of CRM ($8.3 \pm 2.45 / 4$ min.) than PRM ($1.5 \pm 0.3 / 4$ min.) (d.f. = 9, t -critical = 2.262, $P < 0.014$) after 6 h starvation (Fig. 4.5) This corresponds with the highest number of CRM:PRM proportions in the arenas (0.62 and 0.38, respectively). However, SBM deutonymphs only fed on PRM that were encountered (t -critical = 2.262, $P < 0.002$) and did not feed on CRM. Similar results were found for SBM adult females (Fig. 4.5), although in this case the number of CRM and PRM encountered by 6 h starved SBM females (d.f. = 11, t -

critical = 2.200, $P = 0.29$) and 24 h starved SBM females (d.f. = 19, t -critical = 2.093, $P = 0.709$) were not significantly different (t -test, $P > 0.05$) (Fig. 4.5). SBM females fed on 1.8 ± 0.47 and 3.5 ± 0.45 PRM / 4 minutes after 6 h (d.f. = 11, t -critical = 3.10) and 24 h (d.f. = 19, t -critical = 2.86) starvation, respectively, and did not feed on CRM. These results were highly significant ($P < 0.002$ and $P < 2.49^{-7}$, respectively).

Discussion

SBM did not complete its life cycle on the CRM diet. SBM molted from larva to protonymph when provided with CRM alone. However, this was not an indication that SBM larvae were preying on CRM since SBM larva maintained on the ceramic tiles with wet cotton strips molted to the protonymphal stage. This result is similar to previous reports where *I. zuluagai* Denmark and Muma in Brazil, a close relative of *I. quadripilis* did not eat during the larval stage (Yamamoto, 1994). Lee and Davis (1968) found that *Typhlodromus occidentalis* (Nesbitt) larvae did not feed. Sabelis (1985a) noted that the larval stage is not influenced by the prey supply for most phytoseiids. Chittenden and Saito (2001) concluded that the non-feeding larval behavior may be an adaptation to avoid sibling cannibalism. They also gave examples of non-feeding larvae of *Amblyseius californicus* McGregor, *A. degenerans* (Berlese), *A. womersleyi* Schicha, *Phytoseiulus persimilis* Athias-Henriot, and *Typhlodromus bambusae* Ehara.

There were statistical differences in the duration of the SBM egg stage among the different diets (Fig. 4.4). However, the diet did not have an apparent effect on the total developmental times of SBM females as shown in Fig. 4.4. Diets of oak alone and CRm alone were significantly different on the subsequent duration of the egg stage but total developmental times were not significantly different. Significant differences were

obtained in nymphal periods (Fig. 4.4). In this study, the duration of the nymphal stage has a direct relationship with the total duration of the SBM generational time (Fig. 4.4). The shortest nymphal stage was obtained with the TCM diet alone and this was significantly different from the rest of the diets. This short developmental time contributes to the total generation time ($P < 0.05$) of SBM that was also the shortest and statistically significant ($P < 0.05$) from the rest of diets except the oak pollen diet (Fig. 4.4). The longest generational time was with the CReM diet alone and this also affected the total generation time of SBM (Fig. 4.4). Both periods of development with the CReM diet alone were significantly greater ($P < 0.05$) than the rest of the diets tested.

The diets with TCM alone and CReM alone resulted in the longest and shortest developmental times of SBM, respectively. Diets of tetranychids reportedly affect the duration of different phytoseiid life cycles. *Proprioseiopsis aetus* Chant (Phytoseiidae) fed on a diet of *Eutetranychus orientalis* Klein instead of date pollen (*Phoenix dactylifera* L.) and shortened its generation time (Fouly, 1997). Abou-Setta and Childers (1989) fed *Euseius mesembrinus* a diet of ice plant and had the shortest generation time (9.6 d) whereas with CReM it was the longest (11.9 d). These authors indicated that when mated *E. mesembrinus* females were changed from a diet with ice plant to a diet with CReM high mortality resulted. Sabelis (1985a) found that the duration of the nymphal stage and the preoviposition period depend on prey availability. In this case, all the diets provided an abundant food supply, thus differences in developmental time between the tetranychid diets might be explained by two reasons. First, reduced webbing was produced by TCM when compared with CReM. This webbing has been observed disturbing the movement of SBM and increasing the SBM searching area on CReM arenas. Presence of webbing

is explained by Gerson (1985) as a defensive mechanism for CReM to protect spider mite eggs. Abou-Setta (1988) also found that *E. mesembrinus* had similar difficulties when preying on CReM due to the presence of webbing. *Euseius mesembrinus* became entangled in the CReM webbing. Although CReM is not a prolific web producer as is *T. urticae*, the presence of webbing had a repellent effect on SBM because many immatures and adults died trying to leave the arenas. This may explain why the lowest percent survival rate was found with the CReM only diet. The second explanation may be justified by possible differences in nutrients provided by the two types of spider mites. TCM may be considered a better food source than CReM. In Florida, similar results were noted for *E. mesembrinus*. The developmental time was shorter in a diet with TCM than CReM (Abou-Setta and Childers 1989).

No significant differences were found in SBM development times on diets with oak or ice plant pollen alone. However, the survival rates were higher in the pollen diets than with the spider mite diets. SBM may be adapted to feed on pollen and consequently has a preference for pollen because various pollen types are available throughout most of the year in Florida. This also, may be the result of the SBM life history. SBM is a polyphagous predatory mite and it is abundant during the peak pollen season (See Chapters 3 and 4). Muma (1971) described this mite as a fungal feeder and similar behavior was observed during the duration of this study (personal observation). A fungus was observed more frequently on the oak and ice plant pollen arenas than on tetranychid arenas due to deterioration of the pollen. It is possible that SBM had fed on this fungus thus a mixed diet of fungus and pollen can provide an additive nutritional effect for SBM and shortened its life cycle. SBM eggs present a characteristic reddish coloration that is

distinct from other phytoseiids on Florida citrus. SBM that fed on the two pollen diets produced eggs with a small pinkish area instead of an intense, larger reddish area observed on SBM reared on CReM or TCM and from eggs collected on the field. This difference in egg color can be an indicator of the varied diet sources of SBM in the field.

The experiment on predation of citrus rust mites by SBM showed a clear preference by SBM for PRM over CRM as a food source. This preference was obtained for SBM nymphs and adult females starved for 6 and 24 hrs since these instars preyed only on PRM and did not prey upon CRM (Fig. 4.5). Encounters of SBM with citrus rust mites (both PRM and CRM) can be described as direct palpation of rust mites nymphs and adults. In some cases, the rust mites were raised by the pedipalps and/or chelicera of SBM. This behavior may be an indicator of attempted predation but all CRM were left alive after a couple of seconds and, in other cases similar behavior was observed toward the PRM. When SBM preyed on PRM it was observed that the SBM completely sucked the body fluids (haemolymph) from PRM and only left the shrunken exoskeleton. This predatory behavior clarifies previous studies about the feeding of citrus rust mites by SBM. Muma (1971) described that CRM was both inadequate and adequate as a food source for SBM. However, he did not distinguish between CRM and PRM. This latter point may be an inaccuracy in previous studies between predatory phytoseiids and rust mites on citrus worldwide if more than one eriophyid species occurs. The inability of SBM to prey on CRM is similar to responses of *E. mesembrinus* observed by Abou-Setta (1989) and Flores et al. (1996). The reason why SBM did not prey on CRM requires further study and may involve nutritional quality, pheromonal or toxic effects of this mite toward this phytoseiid species. Sabelis (1996) hypothesized that eriophyids had a low

nutritional quality and low profitability compared to other prey. In addition, toxic substances within eriophyids may have a repellent affect toward phytoseiids. One or both of these factors may be especially relevant with CRM. It is necessary to make a clear distinction between the two eriophyids found in citrus orchards in Florida because many earlier studies on predatory mites assumed only the presence of CRM. Childers and Achor (1999) stated that in future studies it is important not to lump the rust mites because their development, biology, feeding damage and seasonal activities obviously differ.

In this study, the effects of the diets on oviposition during the entire life span of SBM were not followed. However, this study has provided valuable information on the identification of various food sources, developmental times and behavioral aspects of this important predatory mite in Florida. We learned that SBM is able to feed and complete its life cycle on pollen, CReM and TCM and, it will prey on PRM but not on CRM. This work enhances the importance of identifying correctly CRM from PRM. The small size of the eriophyids makes it more difficult to identify species with a handlens. The only viable way to effectively distinguish these two species is by the pinkish coloration of the pink citrus rust mite (Childers and Achor 1999). However, this requires practice and most importantly the confirmation by slide-mounted specimens.

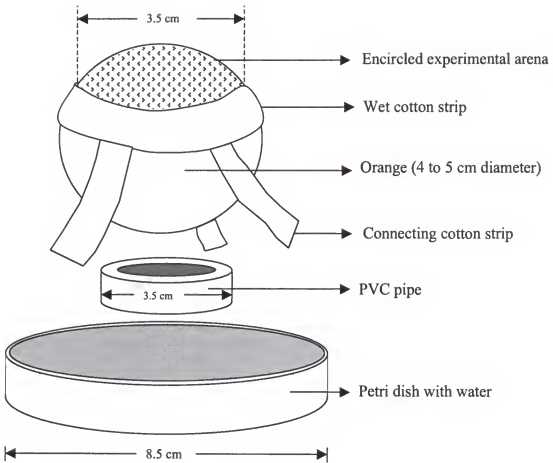


Figure 4.1. Orange fruit arena: design and components.

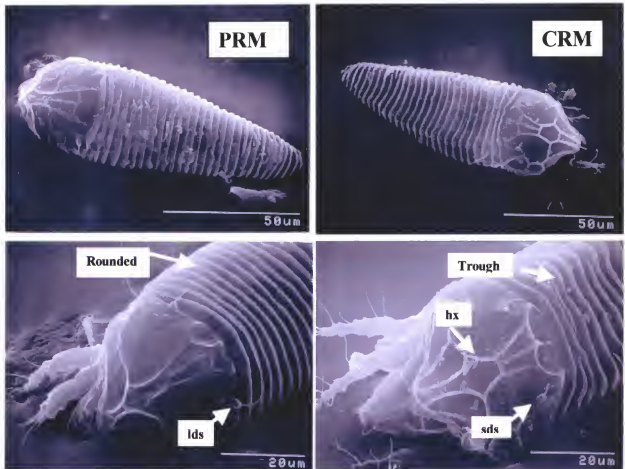


Figure 4.2. PRM (*Aculops pelekassi*) and CRM (*Phyllocoptruta oleivora*) main taxonomic differences. PRM: long dorso-lateral setae (lds), rounded dorsum and distinctive dorsal shield. CRM: short dorso-lateral setae (sds), dorsum with trough present and dorsal shield with well defined hexagon (hx).

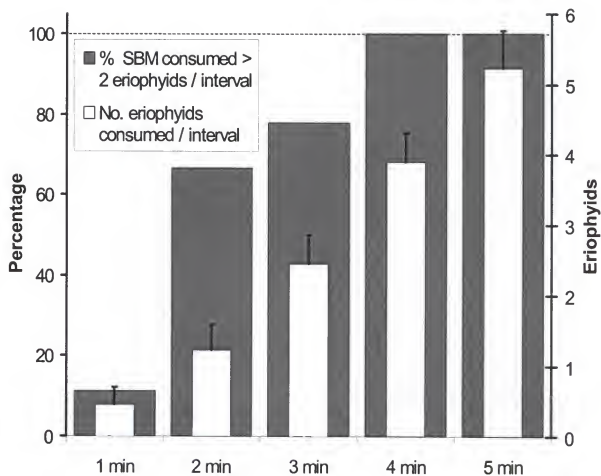


Figure 4.3. Determination of time interval for predation of eriophyids by *Iphiseiodes quadripilis* (SBM)

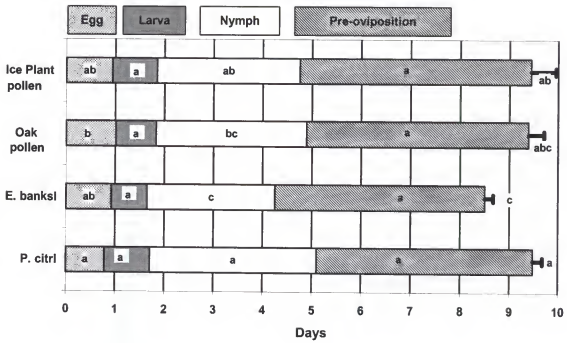


Figure 4.4. Duration of female *Iphiseiodes quadripilis* life stages provided individual diets. Different letter between diets in the same instar indicates significant differences (LSD -test, $P < 0.05$)

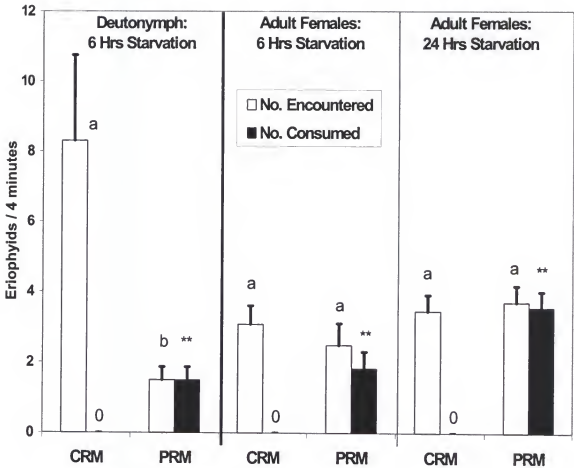


Figure 4.5: Consumed and encountered eriophyids by SBM deutonymphs and adult females after starvation. Different letter between the numbers of eriophyids encountered or consumed within the same time of starvation and instar indicates significant differences (t -test, $P < 0.05$).

CHAPTER 5
EVIDENCE FOR HOST PLANT PREFERENCE IN A PREDACIOUS MITE
IPHISEIODES QUADRIPILIS (ACARI: PHYTOSEIIDAE)

Introduction

Price et al. (1980) proposed the three-trophic level theory that establishes the basis for interaction among herbivores (prey), natural enemies (predators) and their host plants. This theory suggests that natural enemies and plants have a mutualistic relationship. Inherent plant defense mechanisms and plant traits that foster natural enemies evolved through natural selection and both are part of a plant defense that results in a negative impact on herbivores. These traits included attractants (nectars, pollen) and structural refuges (leaf domatia) (Walter 1996, O'Dowd and Willson 1997). Research in plant volatiles demonstrated that plants send signals -plant volatiles or infochemicals – to predators and these predators respond to them. *Phytoseiulus persimilis* Athias-Henriot responded to volatiles emitted by bean plants when they were damaged by punctures that mimicked *Tetranychus urticae* (Koch) feeding damage (Dicke and Sabelis 1988, Takabayashi and Dicke 1992, Dicke et al. 1998). However, all of these observations were on annual plants in the greenhouse. They are less complex than perennial plants in the field due to greater variables such as the presence of other organisms, environmental conditions and space. Drukker et al. (1997) showed the influence of *P. persimilis* performance and adaptation on tomato plants. They found that *P. persimilis* maintained for four generations on tomato exhibited higher survival and reproduction compared to *P.*

persimilis reared on bean plants and then transferred directly onto tomato. They explained that there was an intergenerational adjustment in the behavior and physiology of *P. persimilis* and a selection of *P. persimilis* genotypes with higher fitness on tomato plants. Similarly, Duso and Vettorazzo (1999) showed that two native phytoseiids in Italian vineyards distinctly preferred different grape (*Vitis vinifera* L.) varieties as host plants. *Amblyseius andersoni* (Chant) was more abundant on 'Merlot' grapes that had slight pubescence on the undersurface of its leaves. *Phytoseius finitimus* (Ribaga) was more abundant on 'Prosecco' grapes with a greater under-surface leaf pubescence than glabrous leaves of 'Riesling' grapes.

Iphiseiodes quadripilis is a generalist predatory mite on Florida citrus (chapter 4) that feeds on spider mites, ice plant and oak pollens in the laboratory. However, little is known about its life history and ecology. Abou-Setta et al. (1991) described the post-embryonic stages and noted that the *I. quadripilis* female retained an egg with a well-developed larva. *Iphiseiodes quadripilis* was the most abundant predatory mite on mined leaves of grapefruit damaged by citrus leafminer larvae and exploits this type of leaves better than other phytoseiids (Chapter 3). Observations during my field work led me to assume that *I. quadripilis* tended to be more abundant on grapefruit than on sweet orange trees. Hence, this question was addressed with both field and laboratory assays. This mechanism implies a tritrophic relationship in a perennial plant that needed to be investigated. The aim of this study was to determine if there is a preference by *Iphiseiodes quadripilis* for grapefruit compared with sweet orange leaves.

Materials and Methods

Abundance of *Iphiseiodes quadripilis* on the field.

Four adjacent orchards of grapefruit and sweet orange were selected for this experiment. The pairs of adjacent orchards at the four sites received similar types of cultural practices and pesticide regimes. Three are experimental orchards in the vicinity of the Citrus Research and Education Center at Lake Alfred and the fourth site is a commercial orchard in Highland City (Polk County, FL). The first orchard was Block 1 and trees were 3-year-old 'Lang' navel oranges and 'Marsh' grapefruit. Leaves were collected on 12 and 19 April 2001. The second orchard was Block 24B and had 10 rows of 10 different types of citrus trees (mandarins, oranges, grapefruit), with 1 row per variety and with 8 to 12 trees per row. The trees were planted in 1990. This orchard was adjacent to a large experimental orchard of "Valencia" oranges. The varieties selected here were 'Pineapple' orange and 'Oroblanco' grapefruit and the leaves were collected on 18 and 22 April 2001. The third orchard was Block 22 with 'Hamlin' oranges and 'Flame' grapefruit. Samples were taken on 15 and 20 April 2001 and the trees were planted in 1997. The fourth orchard in Highland City included 20 and 23 years old 'Hamlin' oranges and 'Marsh' grapefruit, respectively. The samples were collected on 17 April and 7 May 2001.

Five border trees of each orange and grapefruit block were selected in each of the sites and 10 mined leaves (mines damaged by the citrus leafminer *P. citrella*) were taken randomly from each tree. Leafminer damaged leaves were selected because they have higher numbers of phytoseiids (see Chapter 3). The cumulative number of phytoseiids was analyzed using the test of proportions Statistica® (Statsoft 2000) to observe if there were differences between the total numbers of phytoseiids found on grapefruit or orange.

A similar test was completed for the total number of *I. quadripilis* and other species of phytoseiids. Also, only data on TCM eggs and motiles, *I. quadripilis* and the total number of phytoseiid motiles found on the two sample dates per citrus species were analyzed separately with a mixed analysis of variance using the Satterthwaite method Statistica® (Statsoft 2000). the data were transformed using $\sqrt{X + 0.5}$, where X is the number of mites counted on 5 leaves. Mean numbers \pm SEM employed in figures and mentioned in the text are untransformed data.

Additional samples were taken from Block 22 on 29 April from the border trees of both orange and grapefruit and phytoseiids counts were completed. The 12 May samples were taken from the border trees and 5th row inside of each type of citrus fruit and counts of both phytophagous and predatory mite eggs and motiles were completed in the laboratory. This time only 5 trees were sampled and 5-mined leaves were taken from each tree. Data were transformed using $\sqrt{X + 0.5}$, where X is the number of mites counted on 5 leaves. Data were analyzed using the analysis of variance for repeated measures followed by a Scheffe-test (Statsoft 2000).

Direct contact choice experiment on 12 by 2 mm leaf strips

Ten female *I. quadripilis* were collected from each orange and grapefruit block and then placed on an arena. The arenas consisted of 3 alternating strips of orange and grapefruit leaves surrounded by wet cotton strips. The strips were 12 mm long by 2 mm wide (Fig. 5.1). Leaves used for the arenas were previously washed thoroughly with tap water and all mites and insects removed. Observations and counts of mite preferences were completed every 5 minutes for 1 h. Three replicates were conducted for each of the types of plants (orange or grapefruit) where *I. quadripilis* were obtained. All of the adult

female *I. quadripilis* were placed in alcohol and later slide-mounted in Hoyer's mounting medium and identified. This experiment was completed in the laboratory at 25° C and 55 ± 5 % RH during May 2001. Data were analyzed using a paired *t*-test for comparison of means (Excel ®, Microsoft).

Y- Tube olfactometer

Iphiseiodes quadripilis females were collected from orange and grapefruit trees on several dates during May 2001. The *I. quadripilis* were collected from orange leaves and then placed on an orange leaf arena. Those collected from grapefruit were placed on a grapefruit leaf arena. Leaves from the same orchards were also collected and returned to the laboratory. These leaves were washed thoroughly with deionized water to remove any prey, scale insects or dust and then air-dried. The Y-tube olfactometer model (Fig. 5.2) was constructed using the design of Sabelis and Van de Baan (1983). Air was pumped at a rate of 500 cm³ of air current per minute and measured by a Gas flowmeter (Dwyer Instruments, Inc. Michigan City, Indiana). The air passed through activated charcoal before entering the odor chambers.

For each plant type, 70 *I. quadripilis* females were employed and used only once. A single female *I. quadripilis* per trial was placed at the mouth of the Y-tube and left there to make an odor attraction choice of leaf. The leaf sources were in the chambers at the ends of the Y-tube arms and consisted of either a 3.5 cm leaf disk of orange or grapefruit. A 5-minute time limit for each predator was provided to make a choice. The assay for each female ended when the predator entered into one of the two chambers. The chambers of the Y-tube were switch every four trials and the 3.5 cm disks were replaced after each switch. Mites that did not make a choice during this time limit were separated and not considered in the statistical analysis. This experiment was completed in the

laboratory at 25° C and 55 ± 5 % RH between May and June 2001. The number of predacious mites that chose either grapefruit or orange were counted as well as the time required in each trial. Data for the choice test were analyzed using the χ^2 test and the time required per predator to reach either chamber was analyzed using the ANOVA (Excel®, Microsoft, 2000).

Results

Abundance of *Iphiseiodes quadripilis* in the field

The total numbers of phytoseiids by type of fruit and species found at the four sample sites during the two collection dates are shown in Table 5.1. Significantly more *I. quadripilis* ($P < 0.001$) mites were found on grapefruit than orange, and the 'other phytoseiids' (phytoseiids other than *I. quadripilis*) were found in higher numbers on orange than in grapefruit ($P < 0.001$). The total numbers of phytoseiids were also significantly higher ($P < 0.001$) on grapefruit than on orange. However, the total numbers of *I. quadripilis* were not different from the total numbers of other phytoseiids ($P > 0.05$).

The data for the two sample dates and four sites gave us the results observed in Tables 5.2 to 5.6. A more detailed analysis of these results is presented in the appendix. *Iphiseiodes quadripilis* was significantly more abundant ($P < 0.05$) on grapefruit than orange (Tables 5.2 and Figs. 5.3 A and B). The highest mean numbers ± (SEM) of *I. quadripilis* motiles on the first date of samples obtained in Block 24B were 20.0 ± 7.7 motiles / 10 leaves on 'Oroblanco' grapefruit versus 2.8 ± 1.4 motiles/ 10 leaves for 'Pineapple' oranges. The lowest mean numbers ± (SEM) of *I. quadripilis* were found at the Bader block with 4.6 ± 1.5 motiles / 10 leaves on 'Marsh' grapefruit and 1.6 ± 1.0 motiles/ 10 leaves on 'Hamlin' oranges (Fig. 5.3A). The highest mean numbers ± (SEM)

of *I. quadripilis* on the second sampling dates were obtained at Block 22 with 22.6 ± 4.2 motiles / 10 leaves on 'Flame' grapefruit versus 5.2 ± 2.8 motiles/10 leaves on 'Hamlin' oranges. The lowest mean numbers \pm (SEM) of *I. quadripilis* motiles were found at the Bader block with 1.8 ± 0.4 motiles / 10 leaves on 'Marsh' grapefruit and 0.2 ± 0.2 motiles/ 10 leaves on 'Hamlin' oranges (Fig 5.3 B).

Phytoseiids other than *I. quadripilis* are referred here as 'other phytoseiids' and their mean numbers \pm (SEM) were significantly different in the first sample dates between orchards and between trees. However, no differences ($P > 0.05$) were found for the second sampling dates for both orchards and tree fruit type (Table 5.3 and Fig 5.3A). Also, no significant differences were obtained between the total numbers of phytoseiids (*I. quadripilis* + "other phytoseiids") found on grapefruit and orange on either of the two sample dates (Table 5.4 and Fig. 5.4). The Texas citrus mite eggs and motiles were very low in the first sampling dates on all orchards except Bader. This was the only significant difference found due to the interaction. There were 9.0 ± 3.9 and 21.0 ± 7.5 eggs and motiles / 10 leaves on 'Marsh' grapefruit and 122.2 ± 41.7 and 140.6 ± 47.4 /10 leaves on 'Hamlin' oranges. No significant differences in TCM egg and motile numbers were found between orchards or fruit types. On the second sampling dates, TCM eggs and motiles were high and significant differences occurred among orchard sites and tree types for the numbers of eggs and between tree types for TCM motiles (Tables 5.5 and 5.6 and Figs. 5A and 5B)

Mean numbers \pm SEM for *I. quadripilis* on samples of 29 April (1,8 df, $F = 37.48$, $P < 0.01$), and 12 May (3,16 df, $F = 46.3$, $P < 0.001$), 10 September (1,8df, $F = 0.80$, $P > 0.05$), and 22 October (3,16 df, $F = 5.68$, $P < 0.01$) from Block 22 are presented in Table

5.7. *Iphiseiodes quadripilis* motiles were more abundant on grapefruit than orange during most of the times sampled except on 10 September. Additional samples of fruit on the fifth row within each orchard gave similar results for *I. quadripilis* motiles but the TCM eggs and motiles were significantly more abundant on orange than grapefruit (Fig. 5.6 A and B).

Direct contact choice experiment on 12 by 2 mm leaf strips

The numbers of *I. quadripilis* females that were collected on grapefruit were significant (11 df, t -critical = 2.20, $P < 0.05$) most of the time on grapefruit than on orange (mean \pm SEM: 5.81 ± 0.18 vs. 4.19 ± 0.18 *I. quadripilis* females in grapefruit or orange, respectively) leaf strips for all replicates (Table 5.8 and Fig. 5.7). Whereas *I. quadripilis* females collected from orange did not show a preference for either orange or grapefruit leaf strips (11 df, t -critical = 2.20, $P > 0.05$) (mean \pm SEM: 4.9 ± 0.26 vs. 5.1 ± 0.26 , respectively) (Table 5.8 and Fig. 5.7).

Y- Tube olfactometer

Iphiseiodes quadripilis females that were collected from orange or grapefruit showed preference for the grapefruit leaf disk. The number of *I. quadripilis* from grapefruit that selected grapefruit and orange were 44 and 26, respectively. By coincidence, the number of *I. quadripilis* females collected from orange that preferred grapefruit and orange were also 44 and 26, respectively. In both cases, these numbers were significantly different (1 df and X^2 critical = 3.84). The times employed for *I. quadripilis* females collected from orange or grapefruit in making their choice for either leaf disk were 115.0 ± 8.91 and 97.6 ± 8.2 seconds (mean \pm SEM), respectively, and these were not significantly different (1, 138 df, $F = 2.06$ and $P = 0.15$).

Discussion

The same species of phytophagous mites affect orange and grapefruit orchards although some citrus species may tend to be more susceptible to certain kinds of pest mites than others. The broad mite *Polyphagotarsonemus latus* (Banks) is more commonly found on lemon and lime in south Florida than on grapefruit (Childers 1994a). However, *I. quadripilis* (SBM) was observed in highest numbers on grapefruit than orange on the border trees of the four adjacent sites. This indicates a preference of *I. quadripilis* for its host plant. This can be inferred because each pair of adjacent orchards received similar cultural practices and pesticide regimes. Furthermore, prey availability was favorable to orange, although in the first sampling dates there were no differences in *E. banksi* (TCM) eggs and motiles with the exception of the Bader orchard. During the second sampling, the densities of TCM were observed to be lower on grapefruit than on sweet orange, suggesting that better biological control by *I. quadripilis* was occurring on the grapefruit varieties. If this was the case, why did *I. quadripilis* not migrate from grapefruit to orange? Is this a seasonal occurrence or are there other factors that retain *I. quadripilis* on grapefruit? There must be since *I. quadripilis* developed and reproduced readily on *E. banksi* (Chapter 4) and this pest mite was present in greater numbers on orange than on grapefruit. Also, *I. quadripilis* had the shortest developmental rate on *E. banksi* compared to *P. citri*, oak (*Quercus* sp) or ice plant (*Mallephora crocea*) pollen diets (Chapter 4).

Thus a mutualistic relationship between grapefruit and *I. quadripilis* appears likely. If a mutualistic relationship exists, it may be independent of prey availability such as an enhancement of the host plant to foster predators. Other unknown factors may exist that contribute to grapefruit plants maintaining *I. quadripilis* populations in grapefruit

orchards. These can be inherent in the plant or exogenous to it or both factors may apply. Inherent factors may include the production of volatiles and a better nutritional quality of grapefruit pollen than orange pollen. Other inherent characters could include the composition of essential oils in leaves of the two citrus species. Cheng and Lee (1981) completed a study of leaf essential oils in ten citrus species including orange and grapefruit. They found 45 different essential oils and the percentage compositions were similar for most except Sabinene and Δ^3 -carene. Sabinene was the most abundant oil in all the species with 49.7% in oranges and 61.9 % in grapefruit and Δ^3 -carene was 4.9% in orange and 0.12% in grapefruit. However, studies of these oils have focused primarily for the perfume and cosmetic industries. It would be interesting to know if one or more of these essential oils affect the composition of mite or insect faunas in the different citrus species.

Exogenous factors may include food sources other than prey mites that are available for *I. quadripilis* such scale insect eggs and/or phylloplane fungi. Muma (1971) found that *I. quadripilis* preyed on whitefly (Aleurodidae) eggs and larvae and described this food source as both adequate and inadequate. However, Muma (1971) did not indicate the reproductive or life cycle parameters of *I. quadripilis* with this prey. No heavy infestations of whitefly were observed in any of the four orchards. Also, it might be possible that some specific types of phylloplane fungi may contribute to the preference of *I. quadripilis* for grapefruit leaves but to this date no studies have been completed on citrus. Effects of phylloplane fungi have been recently studied. Belczewski and Harmsen (2000) found that non-pathogenic phylloplane fungi on beans affect the net reproductive rate of *Tetranychus urticae* Koch. Similarly, Zemec and Prenerova (1996)

observed that the phytoseiid *Typhlodromus pyri* Scheuten feeds on two types of powdery mildew (Ascomycotina: Erysiphales), *Erysiphe orontii* Castellani on tobacco and *Oidium fragariae* Harz on strawberry. Mature females *T. pyri* females successfully reproduced although their oviposition rate was low. Villanueva (unpublished) also observed that *I. quadripilis*, *E. mesembrinus*, *T. peregrinus* and *Amblyseius* sp. feed on unknown fungi that occur within colonies of phytoseiid mites reared in the laboratory.

The contact and olfactometer assays demonstrate that both factors (a grapefruit substrate and volatiles produced by the plant) may contribute to the preference of *I. quadripilis* for grapefruit. In the contact assay, it was apparent that the origin of the *I. quadripilis* population has an imprinting effect on the predator because *I. quadripilis* collected on orange did not show preference for either grapefruit or orange leaf strips. However, *I. quadripilis* collected on grapefruit showed a significant preference for the grapefruit substrate ($P < 0.05$). Imprinting can imply the evolution of two lineages of *I. quadripilis*. This phenomenon was reported on the predatory mite *Hypoaspis aculeifer* (Canestrini) (Acari: Laelapidae) by Lesna (1998) and Lesna and Sabelis (1999). They showed that *H. aculeifer* can rapidly exhibit genetic variation in preference for two prey species: the bulb mite *Rhizoglyphus robini* Claparede or the copra mite *Tyrophagous putrescentiae* (Schrank) depending where *H. aculeifer* was reared. It may be possible that *I. quadripilis* from grapefruit and orange can form two lineages that are very closely related and the only way to differentiate the two would be with molecular analysis rather than by classical taxonomic methods.

Table 5.1 Total number of Phytoseiids found in 4 sites on 2 sample dates by type of fruit and phytoseiid species. Significant differences calculated with the test of proportions are indicated in each column and row

	<i>Iphiseiodes quadripilis</i>	Other Phytoseiids	Total	P
Grapefruit	458	151	609	< 0.001
Orange	114	400	514	< 0.001
Total	572	551	1123	0.25
P	< 0.001	< 0.001	0.007	

Table 5.2. Mixed analysis of variance for *Iphiseiodes quadripilis* numbers found in adjacent rows of grapefruit and orange at 4 sites sampled on two dates each.

	Factor	d.f.	F	P
1 st sample dates	Orchard Site	3,3	2.44	0.241
	Tree type	1,3	15.13	0.030
	Orchard*Tree	3,32	1.37	0.267
2 nd sample dates	Orchard Site	3,3	3.96	0.144
	Tree type	1,3	15.28	0.029
	Orchard*Tree	3,32	2.13	0.115

Table 5.3. Mixed analysis of variance for the total number of 'other phytoseiids' (phytoseiids other than *I. quadripilis*) found in adjacent rows of grapefruit and orange at 4 sites sampled on two dates each.

	Factor	d.f.	F	P
1 st sample date	Orchard Site	3,3	15.19	0.03
	Tree type	1,3	26.08	0.01
	Orchard*Tree	3,32	0.54	0.66
2 nd sample date	Orchard Site	3,3	1.70	0.34
	Tree type	1,3	9.39	0.05
	Orchard*Tree	3,32	0.72	0.54

Table 5.4. Mixed analysis of variance for the total number of phytoseiids found in adjacent rows of grapefruit and orange at 4 sites sampled on two dates each.

	Factor	d.f.	F	P
1 st sample date	Orchard Site	3,3	1.67	0.34
	Tree type	1,3	0.37	0.58
	Orchard*Tree	3,32	0.75	0.52
2 nd sample date	Orchard Site	3,3	3.33	0.17
	Tree type	1,3	3.91	0.14
	Orchard*Tree	3,32	1.17	0.338

Table 5.5. Mixed analysis of variance for the total number of *Eutetranychus banksi* eggs found in adjacent rows of grapefruit and orange at 4 sites sampled on two dates each.

	Factor	d.f.	F	P
1 st sample date	Orchard Site	3,3	2.17	0.26
	Tree type	1,3	1.14	0.36
	Orchard*Tree	3,32	10.65	< 0.001
2 nd sample date	Orchard Site	3,3	16.57	0.02
	Tree type	1,3	23.77	0.01
	Orchard*Tree	3,32	0.52	0.66

Table 5.6. Mixed analysis of variance for the total number of *Eutetranychus banksi* motiles found in adjacent rows of grapefruit and orange at 4 sites sampled on two dates each.

	Factor	d.f.	F	P
1 st sample date	Orchard Site	3,3	2.12	0.24
	Tree type	1,3	4.00	0.14
	Orchard*Tree	3,32	6.54	0.001
2 nd sample date	Orchard Site	3,3	6.00	0.09
	Tree type	1,3	13.79	0.03
	Orchard*Tree	3,32	1.67	0.19

Table 5.7. Analysis of variance for the total number of *Iphiseiodes quadripilis* motiles found in adjacent rows of orange and grapefruit in Block 22 sampled on four dates.

	Orange		Grapefruit		Statistical Test
	5th Row	Border	Border	5th Row	
29 April	-	0 a	11.0 ± 2.9 b	-	ANOVA
12 May	0 a	3.6 ± 1.1 b	13.2 ± 1.4 c	8.4 ± 1.2 c	Scheffe
10 Sep	-	1.1 ± 0.4 a	1.7 ± 0.5 a	-	ANOVA
22 Oct	0 a	1.0 ± 0.6 a	1.7 ± 0.4 b	2.5 ± 0.8 b	Scheffe

Table 5.8 Mean numbers ± SEM of *Iphiseiodes quadripilis* (n = 10) in a choice test between grapefruit or orange strips during 5-minute observations per 1 hour depending on where they were originally collected

<i>Iphiseiodes quadripilis</i>	Replicate	N ^o <i>I. quadripilis</i> on Orange	N ^o <i>I. quadripilis</i> on Grapefruit	d.f.	P
From Grapefruit	1	4.16 ± 0.36	5.83 ± 0.36	11	0.0043
	2	4.41 ± 0.25	5.58 ± 0.25	11	0.046
	3	4.0 ± 0.30	6.0 ± 0.30	11	0.006
From Orange	1	5.25 ± 0.35	4.66 ± 0.35	11	0.49
	2	4.66 ± 0.61	5.33 ± 0.61	11	0.6
	3	4.83 ± 0.36	5.16 ± 0.36	11	0.61

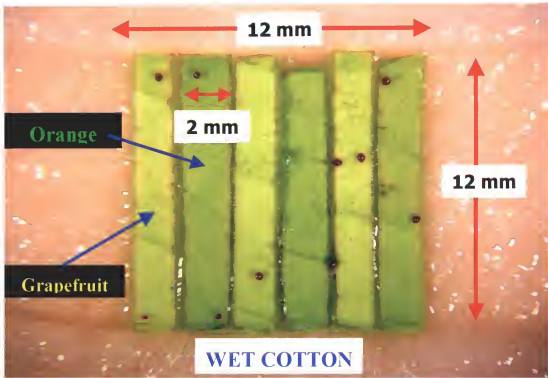


Figure 5.1 Arena used for the contact experiments, Brownish dots on strips are female *Iphiseiodes quadripilis*.

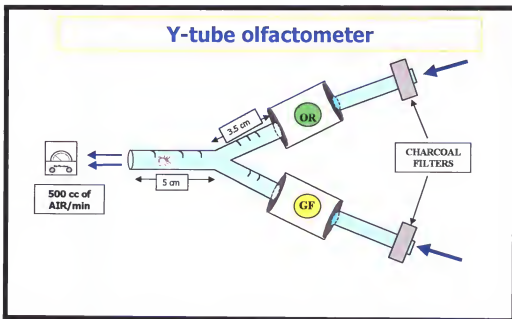


Figure 5.2. Y-tube olfactometer used for the choice test. Leaf disks of grapefruit (GF) or orange (OR) ca. 3.5 cm diameter were the sources of plant volatiles.

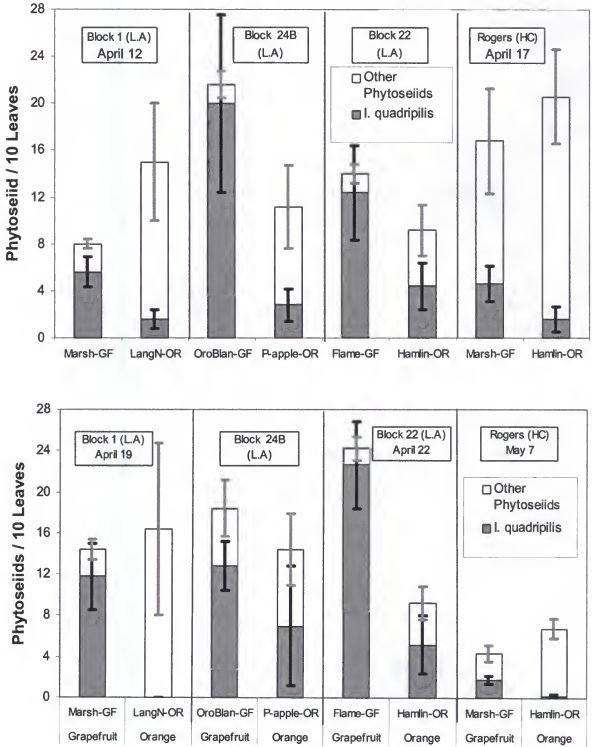


Figure 5.3. Numbers of *I. quadripilis* per 10 leaves and other phytoseiids other than *I. quadripilis*, found on the sample at 4 adjacent orchards of orange and grapefruit on the (A) first sampling dates and (B) second sampling dates.

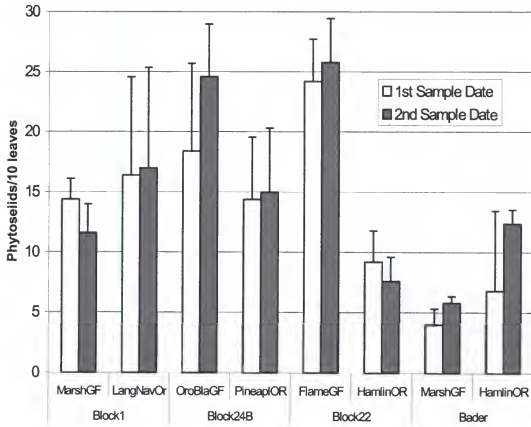


Figure 5.4. Total numbers of phytoseiids (*Iphiseiodes quadripilis* + phytoseiids other than *I. quadripilis*) found on grapefruit and orange in adjacent rows of grapefruit and orange orchards. No significant differences were found ($P > 0.05$).

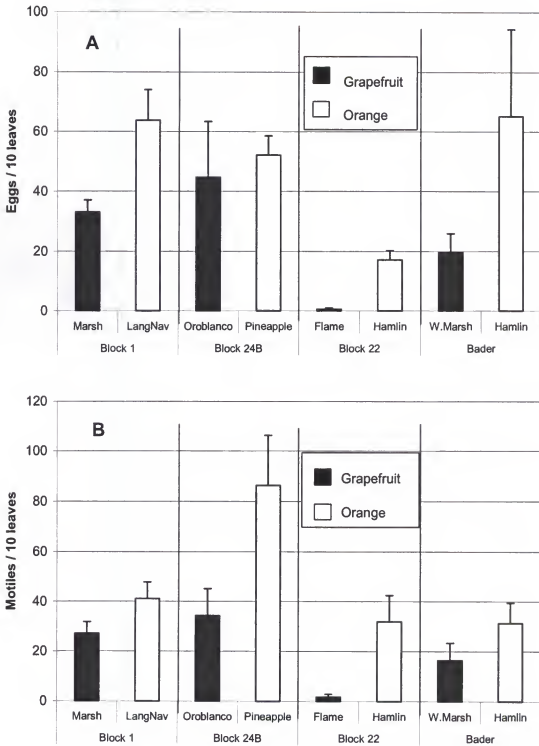


Figure 5.5 Numbers of *Eutetranychus banksi* \pm SEM on the second sampling dates (A) TCM eggs and, (B) TCM motiles

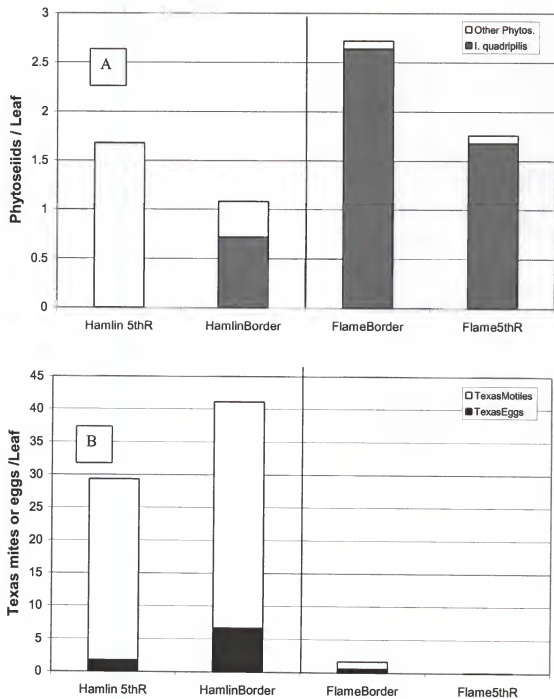


Figure 5.6. (A) Numbers of *I. quadripilis* and other phytoseiids and (B) Numbers of *Eutetranychus banksi* eggs and motiles and eggs found on the 12 May sample on border trees of adjacent grapefruit and orange orchards and 5 rows into each fruit type.

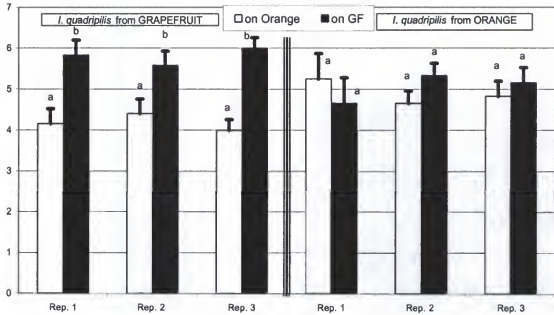


Fig. 5.7 Contact choice test of 10 *Iphiseiodes quadripilis* females for leaf strips of orange or grapefruit. Different letter in each replicate indicates significance ($P < 0.05$)

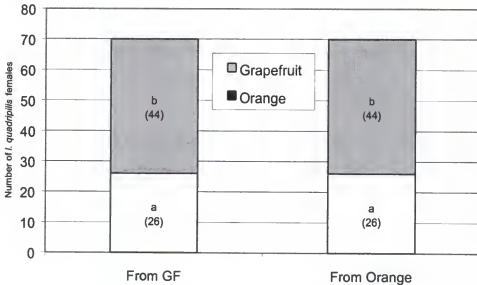


Figure. 5.8. Choice test on leaf disks in the olfactometer for *Iphiseiodes quadripilis*. Each column represents the type of fruit where female *I. quadripilis* were collected. Different letter in each column indicates significance ($P < 0.05$).

CHAPTER 6
ECOLOGY AND FEEDING BEHAVIOR OF TWO NEW SPECIES OF
CECIDOMYIIDAE PREDACIOUS ON RUST MITES AND THE
MICROLEPIDOPTERA *CRYPTOTHELEA GLOVERII* (PSYCHIDAE), A PREDATOR
AND PEST ON FLORIDA CITRUS

Introduction

Two species of rust mites (RM) are pests on Florida citrus, the citrus rust mite (CRM) *Phyllocoptruta oleivora* (Ashmead), and the pink citrus rust mite *Aculops pelekassi* (Keifer) (Acari: Eriophyidae) (Denmark 1963, Childers and Achor 1999). Both species are economically important because they cause fruit russetting. A third species, the citrus bud mite *Aceria sheldoni* (Ewing), occurs on occasion but is not considered an important pest on Florida citrus (Childers and Achor 1999). Natural enemies of citrus rust mites include predatory phytoseiids and stigmatid mites (Muma 1961, Pena 1992, Childers 1994a). Insects of four different orders are also known to prey on citrus rust mites. Hubbard (1883) found "a little coral-red maggot" and a yellow midge larva (Diptera: Cecidomyiidae, formerly Itonididae) feeding on CRM. Later, Muma et al. (1961) reported *Itonidini* species feeding on *P. oleivora* and *Eotetranychus sexmaculatus* (Riley) in Lake Alfred, Florida. Yothers and Mason (1930) described *Stethorus nanus* LeConte (Coleoptera: Coccinellidae) and *Chrysopa* sp. (Neuroptera: Chrysopidae) preying on citrus rust mite and Muma (1958, 1961 and 1967a) mentioned *Coniopteryx vicina* Hagen (Neuroptera: Coniopterygidae) as another predator. The thrips *Scolothrips sexmaculatus* (Pergande) (Thysanoptera: Thripidae) and *Leptothrips mali* (Hunter) (Phlaeothripidae)

were also reported as predators of the citrus rust mite by Bailey (1939) and Muma (1955), respectively.

The genus *Feltiella* has a worldwide distribution (Gagne 1989) and all described species form a distinctive group associated exclusively with spider mites (Tetranychidae) Gagne (1995). This genus belongs to the tribe Lestodiplosini that is composed entirely of predators or parasitoids of insects and mites (Gagne 1989, 1994 and 1995). In Europe, *Feltiella* species are divided into two genera: *Feltiella* and *Therodiplosis*, but Gagne (1995) considered these two genera synonyms. *Feltiella acarisuga* (Vallot) is a well-known predator of *Tetranychus urticae* Koch. *Feltiella acarisuga* completes its life cycle between 8 to 10 d in Italy (Roberti 1954) and 29 d on average in Israel (Sharaf 1984). These differences in the life cycle probably are temperature dependent. *Tetranychus urticae* is a polyphagous pest and has many host plants. The crops included in studies with *F. acarisuga* included apple (Roberti 1954), eggplant (Sharaf 1984), cucumber (Gillespie et al. 1998), strawberry (Easterbrook 1998), greenhouses (Opit et al. 1997, Enkegaard et al. 2000). *Feltiella occidentalis* (Felt) occurs on strawberry in California (Oatman et al. 1985) and *F. minuta* has been found on eggplant (Ho and Chen 1998). There are few examples of eriophyid predation by cecidomyiids. Nijveldt (1969) compiled a list of cecidomyiid species and their respective eriophyid prey. *Medetera* species (Diptera: Dolichopodidae) was reported preying on *Aculus schlechtendali* Nalepa on apple in Washington (Rathman et al. 1988). The eriophyid *Aceria litchii* Keifer is a serious pest of *Litchii chinensis* Sonnerat in Australia and China and a cecidomyiid larva *Arthrocnodax* sp. has been observed preying upon *A. litchii* (Waite and Gerson 1994).

The orange bagworm (OBW) *Cryptothelea gloverii* (Packard) (Lepidoptera: Psychidae) was first described as a hang or drop worm by Glover (1858) in Florida and was originally named *Platoeceticus gloverii* (Hubbard 1885). The bag worms are easily identified because they carry a portable bag of dense silk covered with bits of dry leaves, bark, thorns and other debris pointed outward and fastened to the outside of the case (Quayle 1938). The two ends of the bag are opened; the narrowest opening serves to excrete frass or any other material and later this opening is used for mating. The bag is dragged across the substrate at a certain angle and when the larva moves the head capsule, the thoracic legs are extended from the widest opening of the bag. The bag has an irregular cone shape and enlarges as the larva grows. When full grown the bag is approximately 1.8 and 1.3 cm long for the female and male, respectively (Glover, 1858, Hubbard, 1885). The color of the bag is brownish-gray and is easily camouflaged in the field (personal observation).

In general, species of the Psychidae show sexual dimorphism as adults (Holland 1968, Borror et al. 1989). The adult male is a small, darker moth about 1 cm in length, with legs, well-developed wings and feather-like antennae while the female is maggot-like, wingless, legless, and pupates within its bag (Glover 1858). A common psychid larva that is a defoliator of a wide variety of trees and bushes, evergreens, and reduces aesthetic quality and survival of many ornamental plants is the evergreen bagworm, *Thyridopteryx ephemeraeformis* (Haworth). Leonhardt et al. (1983) described the mating behavior of *T. ephemeraeformis* as an extraordinary event and this behavior may be similar to the OBW. Before pupation, the female *T. ephemeraeformis* attaches and seals the wider part of the bag to a leaf or twig and then places its body with the capsular head upside down, then

pheromone laden hairs are dislodged from the female thorax through the narrow unsealed aperture of the bag that attract males. A male attracted by the pheromone inseminates the female by placing his body upside down and enlarging his abdomen pneumatically through the unsealed opening. The abdomen and aedeagus of the male are extended and pass almost the entire female body length to make contact with the caudal genitalia of the female. The female is known to deposit her eggs within her larval skin, which lines the sack in which she developed (Leonhardt et al. 1983). The female is immediately 'parasitized' by her embryos when they start development within the female body and eventually occupy the entire female body. The female body has been described as being a little more than an ovary (Holland 1968, Kaufman 1968).

Hunt (1923) reported an outbreak of OBW on orange in Sebring, Florida. Damage was observed on fruits, green twigs and mature leaves. The OBW feeds on mature fruit through the flavedo (outer skin) leaving irregular patches of damaged fruit rind. On occasions, the larva penetrates through the epidermis completely but without making entry holes into the fruit sacs. The OBW was described as a predator of various species of scale insects including: the camphor scale *Pseudaonidia duplex* Cockerell; the soft brown scale *Coccus hesperidum* L, a species of *Ceroplastes* and black scale *Saissetia olea* (Bernard) (Plank and Cressman 1934). OBW are distributed throughout the gulf coast states of Texas, Louisiana, and Florida (Neck 1977).

In this chapter the predatory behavior of two cecidomyiid midge species that have been observed preying upon citrus rust mites are described. Also, the ecological relationship of *Cryptothoelea gloverii* (Lepidoptera: Psychidae) with citrus rust mite and orange plants is described.

Materials and Methods

Cecidomyiid Midges Studies

Cecidomyiid collection

Sampling for cecidomyiid larvae was completed in June and from October to the first week of December 2001 in an experimental orchard of 'Hamlin' orange trees at the CREC, Lake Alfred, Florida. Two distinct types of larvae were observed. One was yellow in color (Fig. 2A) and the other was orange with a white collar behind the mouthparts (Fig. 2B). Larvae were collected from citrus leaves and fruits and transferred individually to a Petri dish with a fine 5-0 sable brush. Fruits with high numbers of citrus rust mites ($>100 \text{ cm}^2$) were collected to prepare arenas and to provide a food source for rearing the two midge species. Some of the larvae were allowed to complete their development to obtain adults. Others were used for feeding experiments, behavioral observations and identification of species. Data obtained on individual prey experiments were recorded separately.

Rearing of cecidomyiids

Rectangular transparent plastic containers (Pioneer Plastics Inc #295C, Eagan, MN) with a semi-tight lid (31 cm long by 24 cm wide and 11 cm high) served as rearing chambers for the midge larvae. A lightly moistened piece of paper towel was placed on the bottom of each container to provide increased humidity. 'Hamlin' oranges (4 to 6 cm diam.) with high numbers of CRM were collected in the field and other organisms such as ants, spiders, phytoseiid, stigmatid and tydeid mites were removed. Between 6 to 8 oranges were then placed individually on PVC rings (3.5 cm diam. by 1 cm high) in the rearing chambers and 2 or 3 cecidomyiid larvae were added to each fruit and then the container was covered with the lid. The containers were held in place in the laboratory at

25 ± 1 °C, $60 \pm 10\%$ RH under fluorescent lights set to a day length of 14:10 (L:D). The cecidomyiid larvae were observed daily and oranges with CRM were replaced as required. When a pupa was found it was removed and isolated in a 5 cm diam. Petri dish held under the same environmental conditions. However, detection of pupae was difficult and usually they were not discovered before adult emergency was observed in the rearing chamber. Pupae were found attached to the fruit, on and under the paper towel and on occasions on the plastic walls of the chamber.

Predation by cecidomyiids on citrus rust mites.

The predatory behavior of cecidomyiids was observed in arenas consisting of 'Hamlin' oranges (ca. 4 to 6 cm diam.) containing high numbers of CRM. Individual oranges were placed on PVC rings (3.5 cm diam. by 1 cm high). Numbers of CRM were verified under the dissecting stereomicroscope, and a cecidomyiid larva between 0.6 to 0.8 mm long was placed on top of the orange dome within the center of the field of view of the stereomicroscope. The larva was monitored while rotating the fruit to constantly follow and focus on the midge's movement. The number of eggs, nymphs and adult CRM stages consumed by individual larvae of the two cecidomyiid species were counted separately during 10 min observation intervals. The experiment consisted of 17 yellow and 12 orange cecidomyiid larvae. It was not possible to estimate larval age and all larvae used in the experiment were of similar length between 6 to 8 mm. Data were analyzed with a single factor analysis of variance (Zar 1984).

Orange Bagworm (OBW) *Cryptothoelea gloverii*

Mite predation by OBW

Citrus rust mites predation: Heavily infested 'Hamlin' oranges with CRM were collected in the field and returned to the laboratory. The oranges were ca. 5 to 6 cm diam

and were placed individually on a PVC ring of 3.5 cm diam by 1 cm high. One early instar OBW larva (ca. 0.4 to 0.5 cm bag length) was then placed on top of the fruit to observe its predatory behavior under a dissecting stereomicroscope. To quantify predation of citrus rust mites, 10 'Hamlin' oranges were selected and arenas were prepared as shown in Fig 6.1. This arena consisted of a fruit ca. 5 to 6 cm diam placed on top of a 3.5 cm diam by 1 cm high PVC ring. A 30 ml transparent inverted plastic cup (Jet Plastica Industries Inc., Hatfield, PA) was then placed on top of the upper part of the fruit surface. The 4.0 cm diam plastic cup provided the arena area to assess CRM predation by the OBW and to confine the insect. The area outside the plastic cup was used as a control to quantify CRM numbers without OBW predation. A 1x1 cm square was cut on the base of the plastic cup (opposite to the orange surface) and covered with a fine nylon mesh to maintain adequate moisture. The numbers of CRM were counted on two squares with an area of 1 cm² each within the confined space of the plastic cup. The 1cm² area was defined by 4 dots on each corner of the square marked with a permanent marker on the citrus rind. Citrus rust mites were counted within the limits of the squares. The mean number of CRM in the two 1cm² areas inside the plastic cup perimeter was used as a single replicate for statistical analysis. Correspondingly, two squares with an area of 1 cm² each were marked on the orange surface outside the plastic cup perimeter and the numbers of CRM were counted on each of them. Similarly only the mean of the squares was used as a single replicate for statistical analysis. One OBW per fruit was placed within the enclosed space of the plastic cup and left for 24 h. A total of ten OBW was used in this experiment. The numbers of CRM were counted after this time on the 1cm² areas with and without OBW. All experiments were performed in October 2001 in

the laboratory at $25 \pm 1^\circ \text{C}$, with approximately $50 \pm 5\%$ RH and under fluorescent lights set to a day length of 14:10 (L:D). Data between arenas with and without OBW were compared using the sign test (Zar 1984).

Citrus red mites predation: Two experiments were designed to observe predation by OBW on eggs and adult females of the citrus red mite *Panonychus citri* (McGregor). All experiments were performed in October 2001 in the laboratory at $25 \pm 1^\circ \text{C}$, with approximately 50 % RH and under fluorescent lights set to a day length of 14:10 (L:D). Grapefruit leaves were washed thoroughly and all mites and insects removed. Arenas were prepared using a single mature grapefruit leaf placed on top of a damp sponge pad in a 13 cm diam. plastic Petri dish. The leaf border was covered with a damp cotton strip to avoid mite escape. Each leaf was divided into two sections using a wet cotton strip that crossed the leaf perpendicularly to the leaf midrib. Thirty female *P. citri* were left to oviposit for 36 h on each half then removed and the numbers of *P. citri* eggs were counted. One OBW ($n = 6$) larva was then placed on the arena and egg consumption was assessed after 24 h. The arena for observing predation of *P. citri* females consisted of a grapefruit leaf disk (2.5 cm diam.) with the abaxial side facing up and placed on a wet cotton pad in a 5.5 cm diam. Petri dish. The Petri dish cover had two 0.5 cm diam. apertures for ventilation; each aperture was covered with a fine nylon mesh to prevent escape of the OBW larva. Twenty *P. citri* adult females were placed in each arena and one (ca. 0.5 – 0.6 cm bag length) OBW larva ($n = 6$) was then added to each arena. *Panonychus citri* female consumption was assessed after 12 and 24 h. Consumption here is defined as feeding of the entire *P. citri* female by the OBW. Mites that were found dead or desiccated were not considered consumed because preliminary observations

showed that the OBW completely devoured female *P. citri* without leaving any body parts or exuviae. Mites that were damaged by the movement of OBW were removed and replaced periodically every 4 h. In addition, an orange fruit was observed with a heavy infestation of a scale insect. This fruit was separated and one OBW was placed to determine predation.

Assessment of OBW damage to orange and grapefruit leaves and fruits.

The OBW were found more frequently on russeted orange fruits following CRM feeding injury compared with healthy clean fruits in the field. A choice test assay was designed to evaluate this apparent preference in the laboratory. Rectangular plastic containers (31 cm long by 24 cm wide by 11 cm high) with a semi-tight cover as described for cecidomyiid midges were used as rearing chambers, observations on behavior, fruit damage by OBW and for the preference assay. Pieces of paper towel moistened with deionized water were placed on the bottom to regulate humidity. The preference assay consisted of placing 4 russeted and 4 healthy mature 'Hamlin' orange fruit in two rows within a container as above. The two types of fruit were alternately placed within each row (Fig. 6.2) and then one OBW was placed on each fruit and the damage and location (russeted or healthy fruit) of the larva was observed after 1 and 3 days following infestation. Four replicates were completed and data were analyzed using a paired *t*-test (Ott 1993, Excel® 2000).

Leaf feeding by OBW was observed on 'Hamlin' orange (*Citrus sinensis* [L.] Osbeck), 'Flame' grapefruit (*Citrus paradisi* Macfadyen), 'Volkamer' lemon (*C. volkameriana* Tenore and Pasquale) and trifoliate orange (*Poncirus trifoliata* Rafinesque) in the laboratory using terminal shoots each approximately 8 cm long. The terminals had 4 leaves each and were individually placed in a 50 ml Erlenmeyer Pyrex flask that

contained tap water. A cotton pad was placed as a support to hold the shoots vertically. The shoots were then immediately placed in the rectangular plastic containers of 10 cm long, 13 cm wide and 20 cm tall. Four late instar OBW larvae were placed in each container. Damage to the leaves was observed after 24 h.

Results

Cecidomyiid Midges

Success in rearing cecidomyiids to the adult stage was obtained with larvae collected in November and December, but not from larvae collected in July and August. Dr. Raymond Gagne (USDA, Systematic Entomology Laboratory, Beltsville, Maryland) identified the adult cecidomyiids (Fig. 6.3). Adult males and females of the yellow type larvae were recovered whereas only two females from the orange type larvae were recovered. The two distinct types of larvae (Fig. 6.4 A and B) were identified as belonging in two different genera. The yellow larvae were an undescribed species of *Feltiella* and the females obtained from the orange larvae were identified as *Lestodiplosis* in the broad sense, although they do not really fit in that genus (R. Gagne, personal communication). This would suggest that they represent a new genus of cecidomyiid flies.

Permanent slides mounted with the eriophyids collected during this study were identified as the citrus rust mite *Phyllocoptruta oleivora* (Ashmead). The number of eggs, nymphs and adult citrus rust mites consumed by *Feltiella* n. sp. and the species near *Lestodiplosis* during 10 min. observations are shown on Fig. 6.5. There were no significant differences ($P > 0.05$) for any of the rust mite stages consumed by *Feltiella* n. sp. or the species near *Lestodiplosis*. *Feltiella* n. sp. and the species near *Lestodiplosis* consumed 33.8 ± 4.6 (mean \pm SEM) and 43.0 ± 6.4 ($F = 1.99$; 1,27 df, $P = 0.16$) citrus

rust mite eggs; 14.2 ± 1.4 and 15.0 ± 2.0 ($F = 1.41$; 1,27 df; $P = 0.24$) citrus rust mite nymphs and, 3.0 ± 0.4 and 5.6 ± 0.9 ($F = 0.26$; 1,27 df; $P = 0.60$) citrus rust mite adults, respectively.

Orange Bagworm

Early instars of OBW were observed preying on *P. oleivora* (Fig. 6.6). The OBW larva appeared to be distinctively associated with high populations of citrus rust mites in the field. In the laboratory, the proportion of citrus rust mites decreased significantly on arenas with OBW (0.64 ± 0.07 per cm^2) ($P = 0.02$) whereas citrus rust mites increased in arenas without OBW (1.11 ± 0.08 per cm^2) but they were not significantly different ($P = 0.34$). OBW preying on *P. citri* females was documented on film. OBW consumed 41.8 ± 8.0 (mean \pm SEM) *P. citri* eggs after 24 h and 7.83 ± 1.66 and 10.3 ± 1.0 *P. citri* females after 12 and 24 h, respectively. In the choice experiment between russeted and healthy fruit, significantly more OBW larvae were found on russeted fruits (5.3 ± 0.9) than healthy fruits (2.0 ± 0.7) ($n=4$, $t = 3.18$, $P = 0.03$), respectively, after 24 h (Fig. 6.2). After 3 days, the trend remained significant; more OBW larvae were found on russeted fruits (5.0 ± 0.4) than on healthy fruits (2.25 ± 0.3) ($n=4$, $t = 3.18$, $P = 0.01$), respectively. Visual examination of the fruits in each container showed that more damage was produced on russeted fruits than on clean healthy fruits although numerical measurements of damage were not recorded. Feeding by the OBW larva occurs on the outer epithelial layer of the orange and this was sometimes deep enough to penetrate the rind completely and produce a hole although feeding into the fruit sacs was not observed (Fig. 6.7). The scale insect was identified by Dr. Avas Hamon (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville) as the purple

scale, *Lepidosaphes beckii* (Newman) (Diaspididae) and OBW was observed feeding on the scale. OBW also attached and carried the scale integuments on its bag.

All OBW collected in this study were only found in 'Hamlin' and 'Valencia' orange orchards that were heavily infested by *P. oleivora*. OBW was observed in several counties of Florida (Collier, Hardee, Hillsborough, Lake, Lee, and Polk). It likely is distributed throughout the state in different citrus orchards. Neck (1977) noted that OBW occurs along the Atlantic and Gulf coasts from South Carolina to Central America. However, OBW fed on grapefruit fruits and on young and mature leaves of 'Hamlin' orange, 'Flame' grapefruit, 'Volkamer' lemon and trifoliolate rootstocks in the laboratory after 24 h. Damage on grapefruit is similar to damage observed on orange fruits. OBW feeds on leaves by starting from the border and eating part of the leaf or by chewing holes of 0.3 to 0.8 cm diam. when beginning from the center of the leaf (Fig. 6.8). On occasion, an OBW larva may only consume the outer epithelium of either the axial or abaxial surface of the leaf without penetrating through the leaf. Leaf damage in the field is difficult to identify but it was observed that young flush terminals were eaten at the leaflet borders and in many instances OBW were found near the damaged areas. Conversely, damage to fruit can be easily identified in the field with fruit having one to several holes eaten into the rind or albedo, or fruits may be deformed and flattened on one side due to earlier feeding damage. After initial feeding damage, some citrus fruit were found completely colonized by *Penicillium* sp. (Fungi: Moniliaceae) (Fig. 6.9) and OBW bags were recovered from these fruit. *Penicillium* colonization of fruit was also observed in the laboratory. In addition, OBW larvae were observed feeding on this fungus. Neonate OBW larvae are approximately 1 mm long and their body color was

smoke-beige with the head capsule black. Later instars turn darker to completely black. Male winged adults (Fig. 6.10) were obtained in the laboratory and identified as *Cryptothoelea gloverii* (Packard) (Lepidoptera: Psychidae) by Dr. J.M. Hefner (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville) and Dr. Hugo Kons from the American Entomological Institute in Gainesville. Two male parasitoids of *Chirotica thyridopteryx* (Riley) (Hymenoptera: Ichneumonidae) were recovered from one OBW bag in the laboratory and identified by Dr. Lionel Stange (Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville) and voucher specimens are deposited at the Division of Plant Industry in Gainesville.

Discussion

Cecidomyiid Midges

Predatory reports on *P. oleivora* or other citrus rust mites by *Feltiella n. sp.* and/or other cecidomyiid species on citrus are based largely on anecdotal information. To date, there is not a reliable description of feeding and identification of cecidomyiid predators of citrus rust mites in Florida or any other citrus growing area of the world. McMurtry (1977) and Perring and McMurtry (1996) cited Muma et al. (1961). However, Muma et al. (1961) only mentioned a midge as a predator that was recovered from *P. oleivora* colonies. There were no empirical quantification or scientific descriptions of the feeding behavior. Furthermore, all citations refer to the original descriptions by Hubbard (1883) where he appeared to describe the two types of larvae (one yellowish and the other orange with a white collar) found in this report. In this study, adults of *Feltiella n. sp.* (yellow larva) and a species near *Lestodiplosis* (orange larva with a white collar) were successfully collected, reared and identified to genus. Voucher specimens are deposited

with the USDA, Systematic Entomology Laboratory, Beltsville, MD. In addition, predation by these midges was observed, described and quantified on *P. oleivora*.

Yothers and Mason (1930) and Childers (personal communication) reported that these midges appeared abundant only where high numbers of CRM occurred. This tendency was observed with *F. minuta* when its population increased on *Tetranychus kanzawai* Kishida (Ho and Chen, 1998). It is evident from the consumption data presented here, that *Feltiella* n. sp. and the species near *Lestodiplosis* are efficient predators of *P. oleivora* eggs and nymphs. If we extrapolate the average number of all *P. oleivora* stages consumed in 10 minutes to 24 h then 622 adults, 2,104 nymphs and 5,535 eggs are consumed per day per midge larva. The efficiency of *Feltiella* n. sp. and the species near *Lestodiplosis* surpass the predatory capacity of *Iphiseiodes quadripilis*, when we compare these numbers with the predation rates of *I. quadripilis* on *Aculops pelekassi* (PRM) (Chap. 4). A similar comparison was described with *F. minuta* with respect to *Amblyseius womersleyi* Schicha on eggplant (Ho and Chen 1998).

Laboratory observations of these predators indicate that they are looking for citrus rust mites eggs most of the time, continuously moving the anterior part of the body to the left and then to the right while moving forward and changing direction. Once an egg is detected it is rapidly consumed giving the appearance that the larva is still in search of prey. Consumption of citrus rust mite eggs by these two cecidomyiid species occurs unnoticed by a non-trained acarologist/entomologist due to the small size of an egg (ca. 30-40 μ m) and its whitish to transparent color. All of these observations were taken at 40X magnification using a stereomicroscope. It was noted that the presence of *P. oleivora* eggs are essential for two reasons. First, both types of cecidomyiid larvae were

frequently found on the bottom of the plastic containers and separated from the whole fruit arenas that were still infested with citrus rust mite motiles. However, these arenas lacked citrus rust mite eggs that were already consumed by the cecidomyiid larvae. When a new fruit with abundant citrus rust mite eggs was provided then the cecidomyiid larva immediately started to feed and remained on the fruit. Second, on many occasions, both types of larva encountered adult citrus rust mites but they were not interested in feeding. Other times, they raised the adult citrus rust mite off the substrate with their mouthparts and appeared to throw the motile mite aside. On occasion, when the attacks on citrus rust mite adults were successful, the larval mouthparts were searching or directed to the ventrolateral part of the citrus rust mite body, just behind the second pair of legs. This was not observed when the attacks were directed to nymphs. Nymphs seemed to be more vulnerable and were successfully eaten when grasped on any part of their bodies.

Abundance of cecidomyiids was not recorded in this study because the priority was to identify the midges and evaluate their predation on citrus rust mites. However, most of the larvae collected in the field were more abundant on fruit than leaves during October and November. This apparent abundance of midges on fruits is consistent because citrus rust mites are more abundant on fruits than on leaves especially when fruits are developing as described for *A. pelekassi* (Childers and Achor 1999). Other mites (tydeids, tarsonemids and fungal mites) were present in this orchard too. However, their numbers were low and predation by this midge on either *E. banksi* or *P. citri*, two important tetranychids in Florida may occur. Both mites were essentially absent from this orchard during the time that cecidomyiid larvae were collected.

The search for prey and/or dispersion by these two cecidomyiid species is unclear but it is important to understand their biology and ecology. Observations in both the laboratory and field showed that both species of midges were capable of jumping or springing off the plant substrate. The larva raised the middle part of its body by placing the head to the other end and creating a “loop”. Then it would rapidly let go resulting in a leap or jump (personal observation). The sternal spatula or breastbone aided in this movement as a spring-like device. It is not known if this movement is made at random or if it is directed toward a desired direction. If they fall to the ground, then the larvae would likely desiccate and die. From observations in the rearing arenas in the laboratory, it appears that this movement has a determined direction. When the fruit in the rearing containers had a few citrus rust mite eggs then the cecidomyiid larvae would abandon the fruit and be found on new fruit that had abundant citrus rust mite eggs. The dispersal strategy of these midges and their unnoticed predation on eggs resulted in earlier failed attempts to rear the dipteran larvae. Similar situations have been encountered by Yothers and Mason (1930). They wrote: “These larvae are very small and extremely delicate, and all attempts to rear them to maturity have failed”. Few accurate studies on the biology of predacious cecidomyiids and their effects on prey mite populations are available. This is not only the case with cecidomyiids preying on eriophyids but also on tetranychids (Chazeau 1985).

Orange Bagworm

The OBW was described as a predator of immobile insects or eggs for the first time by Plank and Cressman (1934) in Louisiana, USA. Sands et al. (1986) described a predacious lepidopteran species from South Africa 52 years later that was released for biological control of the white wax scale *Gascardia destructor* (Newstead) in Australia.

Patrick (1999) found the case-bearing larvae of *Rhathamictis perspera* (Meyrick) (Tortricidae) feeding on stationary live prey, including spider eggs, and pupae of *Micromus tasmaniae* (Walker) and *Wesmaelius subnebulosus* Stephens (Neuroptera: Hemerobiidae). Patrick (1999) also observed *R. perspera* feeding on a variety of dead insects in New Zealand. In this study, OBW not only fed on immobile prey such as *L. beckii*, and *P. citri* eggs but also on motile prey such as *P. citri* females.

The predation assays described here with *P. citri* females and *P. oleivora* motiles were artificial because the arenas did not allow mite escape to avoid predation by OBW larvae. If we note the movement of *P. oleivora*, it would be very limited due to its small body size (0.127 mm, Muma, 1975) when compared to the movement of OBW that is about 50 times larger than *P. oleivora*. Thus, it is improbable that *P. oleivora* can escape predation by OBW. OBW larvae decreased the numbers of *P. oleivora* per cm² by 36%. Whereas, *P. citri* egg and female numbers were reduced 68% ($= 41.8 \pm 8.0$) and 50% ($= 10.3 \pm 1.0$), respectively, in the laboratory after 24 h. OBW predation on *E. banksi* eggs and the pink citrus rust mite *A. pelekassi* were not tested. Predation of citrus rust mites can be an adaptation of earlier instars of the OBW for this type of prey due to prey abundance and richer nutrients compared with vegetative tissue. Plant foliage is of low nutritional value compared with insect tissue. For example, the protein content is 2.8 ± 0.6 versus 17.7 ± 1.4 of fresh weight for plant versus insect tissues, respectively (Coll and Guershon 2002). Furthermore, many omnivores enhance their fitness by shortening their developmental times thus reducing their vulnerability to predation (Agrawal and Klein 2000). This latter point needs further study. Neonate OBW larvae that hatched in the laboratory (around middle February) and placed on orange fruit and leaves without rust mites were not able

to survive. This was due either to the absence of rust mites or to inadequate environmental conditions. These conditions were similar to those provided to OBW larvae collected in October 2001 from the field that already had fed on citrus rust mites.

Predation by lepidopteran larvae is a rare event, but there are cases such as the carnivorous caterpillars in the genus *Eupithecia* (Geometridae) from Hawaii (Montgomery 1983 a, b). An *Eupithecia* caterpillar was observed preying for the first time in 1972 by Montgomery. Since then at least 18 distinct species have been identified including *Eupithecia orichloris* Meyrick and *Eupithecia rhodopyra* Meyrick. These predators use different types of perch sites and camouflage to ambush flying and walking prey. The genus *Eupithecia* is distributed worldwide but only the Hawaiian species are carnivores. However, predation by lepidopteran larvae has been described in places other than Hawaii. In the USA, the only predatory lepidopteran larva known is the butterfly *Feniseca tarquinius* (Fabricius) [Lycaenidae]. The female lays eggs in colonies of woolly aphids including the genera *Neoprociphilus*, *Pemphigus*, *Prociphilus*, and *Schizoneura*. The caterpillars prey upon these aphids and sometimes on scale insects, treehoppers and ants (Pierce 1995). In India *Spalgis epius* Westwood, another lycaenid species, has been described as a predator of the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Gowda et al. 1996). OBW is the second example of a predatory lepidopteran found in the USA and the first report of this type of predation occurring between a lepidopteran species and an acarine prey species. Predation of rust mites was observed by neonate larvae (ca. 1 mm) and in later instars (ca. 4 mm) but the effect of this diet on the OBW needs further study.

It is known that some psychid species feed on lichens and other microorganisms (Wessels and Wessels 1991). OBW larval preference for russeted fruit, over clean healthy orange fruit demonstrated in choice test experiments, is consistent with a higher accumulation of microorganisms such as fungi that begin to degrade necrotic epidermis areas on 'Sunburst' mandarin leaves affected by rust mites (Achor et al. 1991). OBW despite its predatory behavior, should be considered a minor pest of citrus because it feeds on leaves and fruit of orange and grapefruit, and the leaves of two citrus rootstocks (Volkamer lemon and trifoliate). It is also possible that this insect feeds on other species of citrus. Leaf damage by OBW larvae was observed in the laboratory on four different hosts, but identifying this type of damage can be a difficult task to recognize in the field because many other insects can cause similar types of damage on leaves. OBW was not very abundant in the field and the search and collection for OBW larvae can also be difficult. They can be found on leaves, branches and fruits and the coloration of the bag can easily be camouflaged with small branches or viewed as a thorn. However, identifying damage on fruit can facilitate detection of bagworms because OBW larvae were generally found either on the damaged fruit or around it. OBW appears to have a low dispersal capacity. Grapefruit damage was obtained only in the laboratory and not a single OBW was found on grapefruit in several fields sampled in the Lake Alfred area. This information coincides with Plank and Cressman (1934) who reported only slight damage on grapefruit in contrast to more severe damage on orange. In the field, fruits damaged by OBW larvae have been observed either deformed or with holes in the albedo. On several occasions, OBW were collected from fruits that were completely infested with the fungus *Penicillium* sp. The low abundance of OBW in the field can be

related to the effect of pesticides or to the presence of parasitoids. The two male parasitoids (*C. thyridopteryx*) that were found on OBW are frequently associated with psyllids and this parasitoid also affects the evergreen bagworm *T. ephemeraeformis* (Bersford and Tsao 1975).

Damage caused by OBW is an open passage for pathogens that can infect fruit more easily and this was observed in both the laboratory and in the field with fruit colonized by a *Penicillium* fungus after feeding injury by OBW. OBW also fed on *Penicillium* and could be categorized as being omnivorous. It was observed that when an OBW larva was feeding on leaves that were perpendicular to the ground, it would be a hard task for the insect to carry the bag and feed. However, OBW attached a part of the bag to the leaf with silk threads before initiating feeding. Thus the larva is able to feed and freely remove most of its body from the case. In the field, an occasional OBW larva was found hanging on trees from silk threads and in the laboratory, neonate OBW were observed in similar situations hanging from the rearing cages. In comparison, neonate larvae of *T. ephemeraeformis* were observed suspended from silk threads and dispersed by ballooning in the wind (Ghent 1999). In the laboratory, OBW larvae were observed laying silk threads in circles around the upper part of oranges within the rearing cages. This behavior may be due to the influence of light or indicates approach of pupation. Debris and frass were observed being excreted through the apical opening of the case. These were small particles of different colors that varied with the food types OBW fed upon.

In this study, three insect species were identified as predators of citrus rust mites. The two cecidomyiids *Feltiella* n sp. and *Lestodiplosis* are new undescribed species and appeared to be effective natural enemies of citrus rust mites. Both appeared highly

specialized in their abilities to eat citrus rust mite eggs and nymphs. It is possible that differences in behavior and prey type exist between the two cecidomyiids. However, the original assumption was that these two types of larvae were one species. Although reduction of citrus rust mite populations for a current season due to predation by the two midges may be unnoticed, the positive effects of these predators may be obtained during the following growing season due to a high reduction in egg numbers. *Cryptothelea gloverii* was long ignored as a pest of citrus but its biology is fascinating. The predatory behavior toward mites remains unknown. OBW is predacious on citrus rust mite, but it is also phytophagous and omnivorous.

To this point, there is not enough data to define correctly how dependent OBW is on citrus rust mites. It has been observed that earlier larval instars of OBW fed on citrus rust mites, but late instars can feed on citrus rust mites due to its omnivorous feeding. Accepting that a citrus rust mite diet is more nutritious and may possibly shorten its developmental time than a plant diet alone, OBW preying on CRM can be adaptively advantageous to improve OBW fitness. Also, any use of OBW to reduce citrus rust mites or *P. citri* populations is minimized by its phytophagy. A positive aspect of its phytophagy is that it prefers russeted to healthy fruit. Consequently OBW cannot be considered a natural enemy of citrus rust mites for biological control purposes but rather a secondary pest of citrus.

Reasons why OBW have not become abundant need to be studied as well as identifying its natural enemies on Florida citrus. Many questions need to be answered about these three insects such as their phenologies, susceptibility to pesticides, dispersal,

and relationships with other organisms. Further studies are needed and should consider alternative prey, host plants, and evaluation of pesticides.

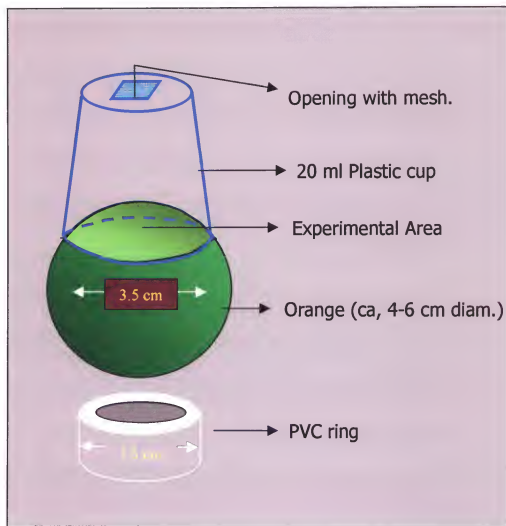


Figure 6.1. Arena used to quantify OBW predation of *Phyllocoptruta oleivora*. The area confined by the transparent plastic cup contained the OBW.



Figure 6.2. Damage caused by *Cryptothelea gloverii* larvae feeding on russeted and healthy 'Hamlin' oranges. *C. gloverii* is more commonly found on russeted fruits.



Figure 6.3. Adult *Feltiella* n. sp. predator of the citrus rust mite *Phyllocoptruta oleivora* obtained in the laboratory, vertical bars beneath insect are in millimeters.

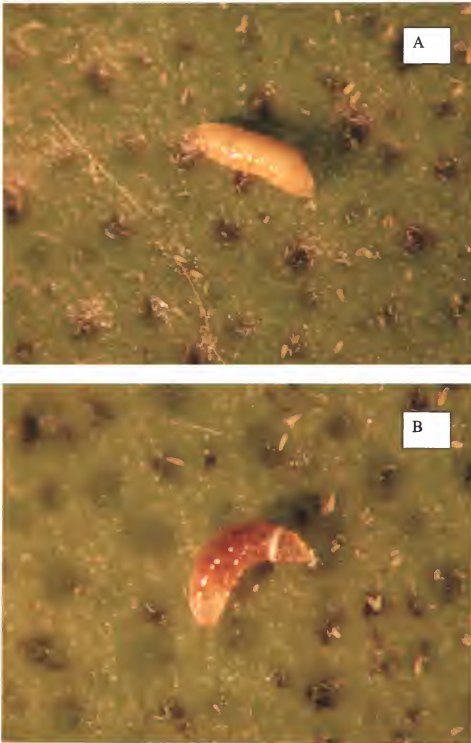


Figure 6.4. Two distinct larval types of two new species of cecidomyiids surrounded by their citrus rust mite *Phyllocoptruta oleivora* prey. (A). *Feltiella* n. sp. and (B) a species near *Lestodiplosis* n. sp. Note ring around the anterior end of the larva.

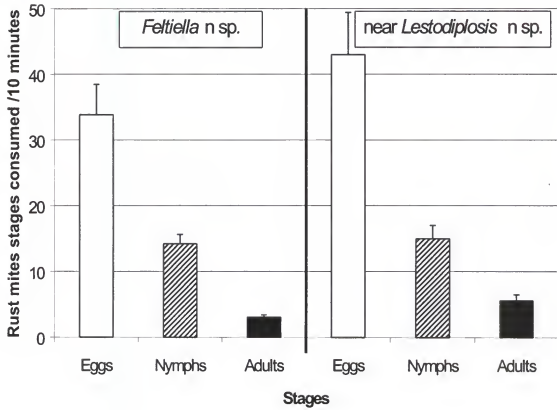


Figure 6.5. Mean numbers \pm SEM of different citrus rust mite (*Phyllocoptruta oleivora*) stages consumed by larvae of *Feltiella* n. sp. and a species near *Lestodiplosis* n. sp.



Figure 6.6. A *Cryptothelea gloverii* larva preying on the citrus rust mite *Phyllocoptruta oleivora*.



Figure 6.7. Hole created by *Cryptothelea gloverii* larvae into the rind of a 'Hamlin' orange. *C. gloverii* larvae penetrate the rind but do not feed on the fruit sacs.



Figure 6.8. Damage produced by a *Cryptothelea gloverii* larva on a citrus leaf. *C. gloverii* can grasp the outer layer of the upper or lower epidermis without penetrating through the leaf and making a hole.



Figure 6.9. Penicillium contamination associated with feeding damage caused by *Cryptothelea gloverii*. The bag of *C. gloverii* (arrow) is often found on or adjacent to this type of fruit and larval feeding on fungi was observed.



Figure 6.10. Winged male *Cryptothelea gloverii*. Scale beside the microlepidopteran is in millimeters.

CHAPTER 7

SUMMARY AND CONCLUSIONS

The 'green revolution' with the use of selected seeds for high yields and modern practices in monoculture, such as use of pesticide sprays against arthropod pests and plant pathogens, and improvement in soil nutrients with chemical fertilizers, has significantly improved crop yields. However, this was only a temporary remedy because phytophagous mite and insect pests have rapidly augmented their populations in these monocultures as a result, in part, due to lower population densities of natural enemies or new pest problems that have emerged. Pesticide resistance development has been an additional problem. Phytophagous mites have this latter response to several pesticides while natural enemies (i.e. phytoseiids) oftentimes are annihilated or greatly reduced because of their lower ability to develop resistance. Hormoligosis in many acarine and insect pests causes increases in their fertility (Voss 1988, Childers 1994b, Omoto et al. 1995). In addition, some pesticides have contributed to contamination of ground water and the environment.

Phytoseiid mites are natural enemies that successfully control phytophagous mite pests in glasshouses in Europe and their predatory capacity has been proven in the field (i.e. on strawberry, Croft et al. 1998, Easterbrook 1998 and on cassava, Yaninek et al. 1998). In citrus, there is a contribution by natural enemies in reducing mite pests, although pest mites are above economic threshold levels. Research needs remain. For example, of the 33 species of phytoseiids found on Florida citrus alone (Childers and Denmark

unpublished) only the biologies of 4 species have been studied in the last 20 years. These species are *Euseius mesembrinus* (Abou-Setta and Childers 1987), *T. peregrinus* (Peña 1992, Fouly et al. 1995), *Galendromus helveolus* (Chant) (Caceres and Childers 1991), and *Proprioseiopsis rotendus* (Abou-Setta et al. 1997). Therefore, research on integrated pest management in orchards and the evaluation of ecological factors of organisms associated with pest control are worthy efforts from both economical and ecological perspectives.

The citrus-mite-insects system is represented in Fig. 7.1. This is a simplified graph without considering pesticides. This system is complex, features such as changes in leaf morphology caused by the citrus leafminer *P. citrella* or plant volatiles are not represented. However, both affect the population composition of mites or small arthropods. The positive effect of mined leaves in providing refuge for phytoseiids was established in Chapter 3, whereas the effects of plant volatiles on *I. quadripilis* were described in Chapter 5. Also, the roles of tydeid mites observed in abundant numbers within citrus orchards are not completely understood. Preliminary reports on their fungivorous habits were recognized by Muma (1975) and the predacious behavior of *Lorryia formosa* Cooreman was identified by Aguilar (2001). Furthermore, the effect of phylloplane fungi on predacious phytoseiids requires further study because some species feed on fungi. These fungi can be an important supplement on their diet breadth when prey are scarce. Antagonistic relationships between natural enemies such as an unknown neuropteran larva preying on a coccinellid pupa or a cecidomyiid larva were observed. Villanueva et al. (2002) observed that *Harmonia axyridis* Pallas (Coccinellidae) consumed *Ambyseius* sp. and *E. mesembrinus* in the laboratory.

In this study, it was shown that three prevalent phytoseiid species in citrus orchards preferred inner leaves of the tree canopy compared with the outer leaves. This is significant to make density evaluations of the three species in the field and increase the accuracy of mite surveys. However, mite movement was not detected in this study from inner leaves to outer leaves during the night as reported by other authors (Muma 1967b, Garcia Mari et al. 1985). The results obtained by Muma (1967a) showed numerical differences in his data but did not mention if they were significantly different. Garcia Mari et al. (1985) mentioned that they sampled only young leaves. It is possible that these young leaves would have had higher numbers of tetranychids eggs and motiles as observed with *E. banksi* during April in this study (Chapter 2). However, young leaves were also observed with low numbers of phytoseiids (Chapter 2).

The high densities of predacious mite populations on mined leaves damaged by *P. citrella* is a novel finding since it has not been previously reported. This can be applied to other crops that are affected by leafminer pests. Villanueva and Harmsen (1997) reported abundant numbers of tarsonemid mites present on apple leaves damaged by the spotted tentiform leafminer *Phyllonorycter blancardella* (Fabricius) (Lepidoptera: Gracillariidae). This implies that leaf distortion caused by other pest species need to be evaluated because they can have a similar positive effect on phytoseiid or other small arthropod populations. One of these organisms is the Asiatic citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) a recently introduced pest into Florida citrus (Anonymous 1999) that produces changes in young terminal leaf flushes.

Higher numbers of phytoseiids were observed on mined leaves than on healthy mature or young leaves (Chapter 3). This result can be useful to facilitate collections of some

phytoseiid species in the field. Searching for phytoseiids on mined leaves is still time consuming because it is necessary to tease apart the rolled or distorted leaves, open the mines and then examine the leaves under the dissecting stereomicroscope.

Comparatively similar numbers of phytoseiids species were recovered from 10-mined-leaves compared to a random 40-leaf sample collected into alcohol jars (See Chapters 2 and 3) during March 1999 and 2001.

Research completed on the shiny button mite (SBM) *Iphiseiodes quadripilis* in Chapters 4 and 5 showed important findings on its biology, behavior and ecology. SBM was able to feed and reproduce on *M. crocea* (ice plant) and *Quercus* sp. (oak) pollens and on different stages of *E. banksi* and *P. citri*. *Iphiseiodes quadripilis* has the fastest developmental time when provided with *E. banksi* as a food source. With *Quercus* sp. pollen, more adults were obtained than the rest of the evaluated diets. It is apparent that both *I. quadripilis* females and larvae did not affect citrus rust mite, *P. oleivora* populations, but selectively preyed on the pink citrus rust mite, *A. pelekassi*. This finding is the first report showing a preference by a phytoseiid species on Florida citrus for one eriophyid species over another. Additional studies with other phytoseiids and non-phytoseiid predators are needed. Preliminary studies with *E. mesembrinus* and an undescribed species of *Amblyseius* in the laboratory resulted in similar outcomes (Villanueva unpublished). The reasons for the rejection of *P. oleivora* are unknown. They may be related with low nutritional quality and toxic substances within *P. oleivora* (Sabelis 1996) and this can be one of the reasons to explain the high abundance of this pest in the field. *Iphiseiodes quadripilis* was observed foraging during daylight hours and this is opposite of reports by Muma (1975) and Muma and Denmark (1971) who found

that *I. quadripilis* was more active after dusk. Also, Muma (1971, 1975) briefly mentioned that this mite was more abundant on grapefruit, but he did present supportive data. In this study, the preference of *I. quadripilis* for grapefruit leaves over orange leaves was demonstrated. The relative abundance of *I. quadripilis* in grapefruit orchards in the field was shown and supported in laboratory assays with direct contact and olfactometer tests to support this argument. This preference needs further study and can be explained by the emission of volatiles by grapefruit that attract and retain *I. quadripilis* in grapefruit orchards despite more abundant prey available in adjacent orange orchards. The establishment of an *I. quadripilis* colony in the laboratory failed in spite of many attempts using single or mixed diets of pollen, spider mites, and honey. Earlier efforts by M. Abou-Setta met with similar problems (Childers, personal communication). However, it has been reported that *I. quadripilis* preyed on eggs of whiteflies and scale insects (Muma 1971). In addition, preliminary assays in this study with *Penicillium* sp. and two citrus pathogens showed that *I. quadripilis* would feed on fungi (Villanueva unpublished). The tritrophic relationship among plants, herbivores and natural enemies is important and complicated as is shown here. As new research becomes available, the system in Fig 7.1 will become increasingly more understandable.

The two cecidomyiids studied here, *Feltiella* n. sp. and the species near *Lestodiplosis* showed that they effectively prey on citrus rust mite eggs, nymphs and adults. They may also prey on *A. pelekassi*. However, *A. pelekassi* were not present during the time of the study. Further studies are needed to know if *Feltiella* n. sp. and the species near *Lestodiplosis* prey upon *P. citri* or *E. banksi* or if they are specific predators of both citrus rust mite species. From this study, we can infer that these midges can cause citrus rust

mite population reductions during the next generation; although Muma (1971) and McMurtry (1977) suggested that cecidomyiids require high densities of citrus rust mites. Compared to the phytoseiids studied, these midges fed voraciously on *P. oleivora* and potentially the numbers of *P. oleivora* motiles and eggs consumed per day per midge could be in the thousands, although this is an extrapolation based on 10 minute feeding studies. It can be argued that phytoseiids are more abundant than cecidomyiids and thus they contribute to the reduction of *P. oleivora* numbers. Yet, these two types of natural enemies and others can have an additive effect in reducing both species of citrus rust mites and should be included in developing integrated pest management practices.

The orange bagworm, *Cryptothoele gloverii* was found to be associated with *P. oleivora* and it appears that this is an omnivorous insect. *Cryptothoele gloverii* preying on either *P. oleivora* or *P. citri* has not been previously reported although *C. gloverii* was observed preying upon scale insects (Plank and Cressman 1934). The impact of *C. gloverii* on *P. oleivora* populations can be minimal because very low numbers of this insect were found. However, *P. oleivora* may have a positive impact on the fitness of *C. gloverii* especially on the first larval instar. This needs further research. *Cryptothoele gloverii* fed on leaves of different varieties of citrus included grapefruit and orange. However, *C. gloverii* were only found on orange orchards with high densities of citrus rust mites across several counties of central Florida counties. The use of *C. gloverii* for biological control in citrus can be inadequate due to the phytophagous behavior of the insect and its ability to damage fruit and leaves. Fruit damaged by *C. gloverii* were easily colonized by pathogens in the field and laboratory.

All of these relationships described above can be observed in Fig. 7.2. This figure focuses on phytoseiids and other organisms that can enhance their populations or be antagonistic to them. These relationships are complex and some of them need further study. For example, Kumar and Mishra (1991) observed that pollen grains on rice favored the development of both pathogenic and saprophytic fungi. Belczewski and Harmsen (1997) sprayed an apple orchard with an epiphytic fungus that favored development of *T. urticae* whereas low numbers of *P. citri* were observed. The antagonistic relationship of spider mites and eriophyids also was observed on both citrus and apple. Finally, plant volatiles have been studied recently and the effects appear favorable toward some natural enemies. Here *I. quadripilis* was observed remaining on grapefruit in greater numbers than on orange despite greater *E. banksi* abundance on the orange varieties. It is possible that plant volatiles not only attract *I. quadripilis* but also retain the predator.

In this thesis we observed that:

1. Phytoseiids preferred inner leaves of the tree canopy to outer leaves.
2. Leaves damaged by the leafminer *P. citrella* are used as refugia for phytoseiid mites and these leaves had higher numbers of phytoseiids compared with healthy mature leaves or young leaves.
3. A high correlation was found between phytoseiid abundance and pollen grain counts on grapefruit leaves during flowering.
4. *Iphiseiodes quadripilis* developed and reproduced on pollens of *M. crocea* and *Quercus* sp and on *E. banksi* and *P. citri*. After starvation *I. quadripilis* only preyed on *Aculops pelekassi* but did not prey on *Phyllocoptruta oleivora*.

5. *Iphiseiodes quadripilis* numbers were more abundant on grapefruit than orange leaves in the field and this mite preferred grapefruit over orange based on olfactometer choice tests. However, the plant type where *I. quadripilis* was collected appeared to have an imprint influence on the type of plant the predatory mite chose in the contact choice test.
6. *Feltiella* n. sp. and a species near *Lestodiplosis* were identified and reared on *P. oleivora*. *Feltiella* sp. larva is yellow in color and the species near *Lestodiplosis* is orange with a white band close to the mouthparts. They prey on eggs, nymphs and adults of *P. oleivora*.
7. *Cryptothelea gloverii* (Psychidae), a microlepidopteran, was found in orange orchards. It preyed on *P. oleivora* motiles and *P. citri* eggs and females but also caused feeding damage on both citrus fruit and leaves.

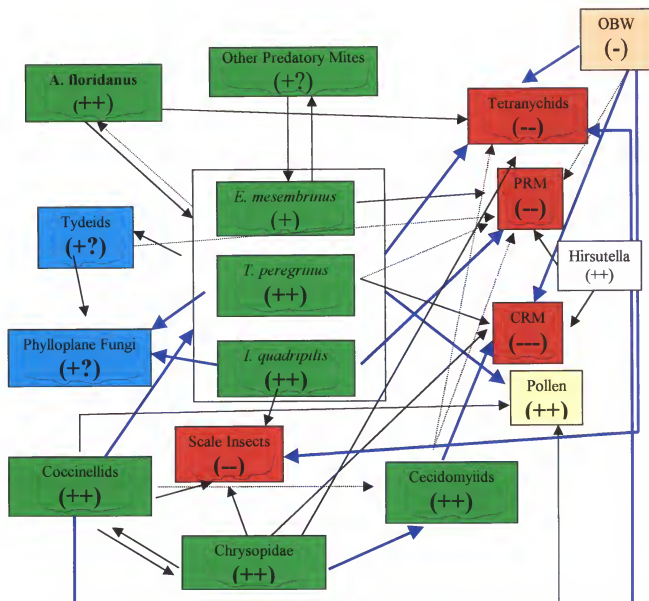


Figure 7.1. Prey-predator relationships of the citrus-acari-insects system on Florida citrus. Predation is indicated by arrows. Broken lines are not well studied relationships, blue lines are relationships observed in this study. (Green) = Natural enemies, (Red) = Phytophagous, (Light blue) = Undefined roles, () = omnivores, and () = pollen. The (+) and (-) signs indicate the relationship to plants. (?) indicates a poorly studied relationship.

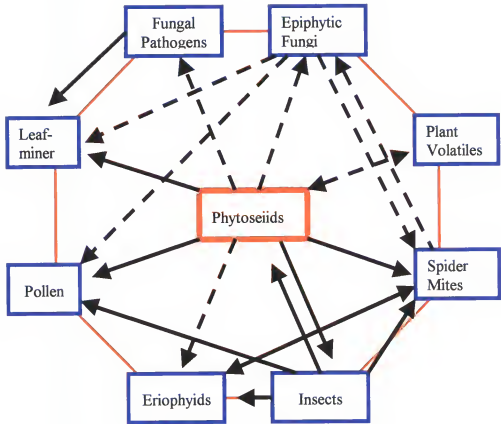


Figure 7.2. Ecological relationships that enhance focusing on phytoseiid mites on citrus. Various relationships were observed in this study. Some were observed in other studies on different crops. Solid black arrows indicate relationships observed in this study. Broken line arrows indicate relationships that require further study. Red line indicates the presence of these organisms on citrus leaves.

APPENDIX
MIXED ANALYSIS OF VARIANCE OF MITES FOUND IN 4 SITES WITH
ADJACENT ORCHARDS OF ORANGE AND GRAPEFRUIT

Table A.1. Transformed means, standard error of means (SEM), and \pm 95% confidence interval limits for total numbers of *Iphiseiodes quadripilis* motiles

1 st sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.30	0.16	1.97	2.64	
{1}Block 1	B1		10	1.87	0.33	1.19	2.54	
{1}Block 24B	B24B		10	2.93	0.33	2.25	3.60	
{1}Block22	B22		10	2.71	0.33	2.04	3.38	
{1}Bader	BAD		10	1.71	0.33	1.04	2.38	
{2}Grapefruit	GF		20	3.06	0.23	2.58	3.53	b
{2}Orange	OR		20	1.55	0.23	1.08	2.03	a
1*2	B1	GF	5	2.40	0.47	1.46	3.35	
1*2	B1	OR	5	1.33	0.47	0.38	2.27	
1*2	B24B	GF	5	4.23	0.47	3.28	5.18	
1*2	B24B	OR	5	1.62	0.47	0.67	2.57	
1*2	B22	GF	5	3.42	0.47	2.48	4.37	
1*2	B22	OR	5	1.99	0.46	1.05	2.94	
1*2	BAD	GF	5	2.16	0.46	1.21	3.10	
1*2	BAD	OR	5	1.26	0.46	0.31	2.21	
2 nd sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.35	0.17	2.01	2.69	
{1}Block 1	B1		10	2.05	0.33	1.37	2.72	
{1}Block 24B	B24B		10	2.81	0.33	2.14	3.49	
{1}Block22	B22		10	3.38	0.33	2.71	4.06	
{1}Bader	BAD		10	1.16	0.33	0.48	1.84	
{2}Grapefruit	GF		20	3.30	0.23	2.82	3.78	b
{2}Orange	OR		20	1.40	0.23	0.93	1.88	a
1*2	B1	GF	5	3.39	0.47	2.43	4.34	
1*2	B1	OR	5	0.71	0.47	-0.25	1.66	
1*2	B24B	GF	5	3.57	0.47	2.61	4.52	
1*2	B24B	OR	5	2.06	0.47	1.10	3.01	
1*2	B22	GF	5	4.72	0.47	3.77	5.68	
1*2	B22	OR	5	2.04	0.47	1.09	3.00	
1*2	BAD	GF	5	1.51	0.47	0.55	2.47	
1*2	BAD	OR	5	0.81	0.47	-0.14	1.77	

Table A2. Transformed means, standard error of means (SEM), and \pm 95% confidence interval limits for total numbers of other phytoseiid motiles (phytoseiids other than *I. quadripilis*)

1 st sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.53	0.23	2.07	2.99	
{1}Block 1	B1		10	2.59	0.43	1.62	3.57	a
{1}Block 24B	B24B		10	1.99	0.41	1.05	2.93	ab
{1}Block22	B22		10	1.70	0.30	1.01	2.38	a
{1}Bader	BAD		10	3.85	0.38	2.99	4.70	b
{2}Grapefruit	GF		20	1.91	0.26	1.36	2.46	a
{2}Orange	OR		20	3.16	0.32	2.49	3.82	b
1*2	B1	GF	5	1.68	0.13	1.33	2.04	
1*2	B1	OR	5	3.50	0.64	1.73	5.27	
1*2	B24B	GF	5	1.25	0.37	0.23	2.27	
1*2	B24B	OR	5	2.73	0.60	1.06	4.40	
1*2	B22	GF	5	1.33	0.29	0.52	2.14	
1*2	B22	OR	5	2.07	0.51	0.65	3.48	
1*2	BAD	GF	5	3.37	0.58	1.75	4.99	
1*2	BAD	OR	5	4.33	0.43	3.14	5.53	
2 nd sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.19	0.20	1.78	2.59	
{1}Block 1	B1		10	2.58	0.61	1.20	3.96	
{1}Block 24B	B24B		10	2.39	0.38	1.53	3.25	
{1}Block22	B22		10	1.62	0.27	1.00	2.24	
{1}Bader	BAD		10	2.16	0.22	1.67	2.66	
{2}Grapefruit	GF		20	1.70	0.19	1.29	2.10	
{2}Orange	OR		20	2.68	0.32	2.01	3.34	
1*2	B1	GF	5	1.64	0.32	0.75	2.53	
1*2	B1	OR	5	3.52	1.06	0.56	6.47	
1*2	B24B	GF	5	2.18	0.58	0.58	3.79	
1*2	B24B	OR	5	2.60	0.54	1.10	4.09	
1*2	B22	GF	5	1.28	0.34	0.35	2.22	
1*2	B22	OR	5	1.96	0.41	0.83	3.09	
1*2	BAD	GF	5	1.69	0.25	1.01	2.37	
1*2	BAD	OR	5	2.63	0.21	2.05	3.22	

Table A3. Transformed means, standard error of means (SEM), and \pm 95% confidence interval limits for total numbers of phytoseiid motiles (*. quadripilis* + other phytoseiids).

1 st sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt
Total			40	4.84	0.24	4.35	5.32
{1}Block 1	B1		10	4.46	0.48	3.49	5.43
{1}Block 24B	B24B		10	4.92	0.48	3.94	5.89
{1}Block22	B22		10	4.41	0.48	3.44	5.38
{1}Bader	BAD		10	5.56	0.48	4.59	6.53
{2}Grapefruit	GF		20	4.96	0.34	4.28	5.65
{2}Orange	OR		20	4.71	0.34	4.02	5.40
1*2	B1	GF	5	4.09	0.67	2.71	5.46
1*2	B1	OR	5	4.83	0.67	3.46	6.20
1*2	B24B	GF	5	5.48	0.67	4.11	6.86
1*2	B24B	OR	5	4.35	0.67	2.98	5.72
1*2	B22	GF	5	4.75	0.67	3.38	6.12
1*2	B22	OR	5	4.06	0.67	2.69	5.43
1*2	BAD	GF	5	5.52	0.67	4.15	6.90
1*2	BAD	OR	5	5.59	0.67	4.22	6.97
2 nd sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt
Total			40	4.54	0.21	4.10	4.97
{1}Block 1	B1		10	4.63	0.43	3.76	5.50
{1}Block 24B	B24B		10	5.20	0.43	4.33	6.07
{1}Block22	B22		10	5.00	0.43	4.13	5.87
{1}Bader	BAD		10	3.32	0.43	2.45	4.19
{2}Grapefruit	GF		20	5.00	0.30	4.38	5.61
{2}Orange	OR		20	4.08	0.30	3.46	4.69
1*2	B1	GF	5	5.03	0.60	3.80	6.26
1*2	B1	OR	5	4.22	0.60	2.99	5.45
1*2	B24B	GF	5	5.75	0.60	4.52	6.98
1*2	B24B	OR	5	4.65	0.60	3.42	5.88
1*2	B22	GF	5	6.01	0.60	4.78	7.24
1*2	B22	OR	5	4.00	0.60	2.77	5.23
1*2	BAD	GF	5	3.20	0.60	1.97	4.43
1*2	BAD	OR	5	3.44	0.60	2.21	4.67

Table A.4. Transformed means, standard error of means (SEM), and $\pm 95\%$ confidence interval limits for total numbers of *Eutetranychus banksi* eggs.

1 st sample dates	Factor	Factor	n	mean	SEM_	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.45	0.29	1.87	3.03	
{1}Block 1	B-1		10	0.71	0.57	-0.46	1.87	
{1}Block 24B	B-24B		10	1.69	0.57	0.52	2.85	
{1}Block22	B-22		10	0.87	0.57	-0.29	2.04	
{1}Bader	BAD		10	6.53	0.57	5.36	7.69	
{2}Grapefruit	GF		20	1.45	0.40	0.63	2.27	
{2}Orange	OR		20	3.44	0.40	2.62	4.27	
1*2	B1	GF	5	0.71	0.81	-0.94	2.35	a
1*2	B1	OR	5	0.71	0.81	-0.94	2.35	a
1*2	B24B	GF	5	1.66	0.81	0.01	3.30	a
1*2	B24B	OR	5	1.71	0.81	0.07	3.36	a
1*2	B22	GF	5	0.71	0.81	-0.94	2.35	a
1*2	B22	OR	5	1.03	0.81	-0.61	2.68	a
1*2	BAD	GF	5	2.73	0.81	1.09	4.38	a
1*2	BAD	OR	5	10.32	0.81	8.67	11.97	b
1*2	B1	GF		2.45	0.29	1.87	3.03	a
2 nd sample dates	Factor	Factor	n	mean	SEM_	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	5.43	0.33	4.75	6.11	b
{1}Block 1	B1		10	6.84	0.67	5.48	8.21	b
{1}Block 24B	B24B		10	6.66	0.67	5.30	8.03	b
{1}Block22	B22		10	2.57	0.67	1.20	3.93	a
{1}Bader	BAD		10	5.65	0.67	4.28	7.01	b
{2}Grapefruit	GF		20	4.24	0.47	3.28	5.21	a
{2}Orange	OR		20	6.62	0.47	5.65	7.58	b
1*2	B1	GF	5	5.77	0.95	3.84	7.70	
1*2	B1	OR	5	7.92	0.95	5.99	9.84	
1*2	B24B	GF	5	6.12	0.95	4.19	8.05	
1*2	B24B	OR	5	7.21	0.95	5.28	9.14	
1*2	B22	GF	5	0.99	0.95	-0.94	2.91	
1*2	B22	OR	5	4.15	0.95	2.22	6.08	
1*2	BAD	GF	5	4.10	0.95	2.18	6.03	
1*2	BAD	OR	5	7.19	0.95	5.26	9.12	

Table A.5. Transformed means, standard error of means (SEM), and $\pm 95\%$ confidence interval limits for total numbers of *Eutetranychus banksi* motiles

1 st sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	2.82	0.32	2.16	3.47	
{1}Block 1	B1		10	1.62	0.46	0.69	2.54	
{1}Block 24B	B24B		10	4.02	0.46	3.09	4.95	
{1}Block22	B22		10	0.71	0.64	-0.61	2.02	
{1}Bader	BAD		10	1.87	0.64	0.56	3.18	
{2}Grapefruit	GF		20	0.98	0.64	-0.33	2.29	
{2}Orange	OR		20	7.71	0.64	6.40	9.02	
1*2	B1	GF	5	0.71	0.91	-1.15	2.56	a
1*2	B1	OR	5	0.81	0.91	-1.05	2.67	a
1*2	B24B	GF	5	0.81	0.91	-1.05	2.67	a
1*2	B24B	OR	5	4.13	0.91	2.28	5.99	a
1*2	B22	GF	5	0.71	0.91	-1.15	2.56	a
1*2	OR	B-24B	5	2.93	0.91	1.07	4.79	a
1*2	OR	B-22	5	1.15	0.91	-0.71	3.01	a
1*2	OR	Bader	5	11.28	0.91	9.43	13.14	b
2 nd sample dates	Factor	Factor	n	mean	SEM	- 95 % Cnf.Lmt	+ 95% Cnf.Lmt	
Total			40	5.28	0.27	4.74	5.83	
{1}Block 1	B1		10	5.78	0.54	4.69	6.87	
{1}Block 24B	B24B		10	7.35	0.54	6.26	8.45	
{1}Block22	B22		10	3.37	0.54	2.28	4.47	
{1}Bader	BAD		10	4.62	0.54	3.53	5.71	
{2}Grapefruit	GF		20	4.00	0.38	3.22	4.77	a
{2}Orange	OR		20	6.57	0.38	5.79	7.34	b
1*2	B1	GF	5	5.20	0.76	3.65	6.74	
1*2	B1	OR	5	6.37	0.76	4.82	7.91	
1*2	B24B	GF	5	5.63	0.76	4.08	7.18	
1*2	B24B	OR	5	9.08	0.76	7.53	10.62	
1*2	B22	GF	5	1.35	0.76	-0.19	2.90	
1*2	B22	OR	5	5.39	0.76	3.84	6.94	
1*2	BAD	GF	5	3.80	0.76	2.26	5.35	
1*2	BAD	OR	5	5.44	0.76	3.89	6.98	

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BIOGRAPHICAL SKETCH

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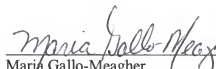
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